Volume 38 *Number 5*

JUN 9 1948
Science Library

Phytopathology

AN INTERNATIONAL JOURNAL

May 1948

Official Organ of

The American Phytopathological Society

$7.75 per year, Canada $7.50 per year, United States and dependencies $8.00 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph 4, Sec. 538, P. L. and R. Authorized May 12, 1921.

TABLE OF CONTENTS

Fusarium diseases of broad bean. II. Further studies on broad bean wilt caused by Fusarium avenaceum var. fabae.
T. F. Yu and C. T. Fang 331

Phoma rot of garden beets ..... Barbara C. Heiberg and G. B. Ramsey 343

Angular leaf spot of kudzu caused by a new species of Mycosphaerella
J. L. Weimer and E. S. Luttrell 348

Canker of tung trees caused by Physalospora rhodina
John R. Large 359

A leaf-curl disease of tomato ..... R. S. Vasudeva and J. Sam Raj 364

Inoculation experiments with Polyporus schweinitzii ... Sigmund Berk 370

White rot of shallot ..... E. C. Tims 378

Infectious chlorosis of Phenax sonneratii ..... Karl M. Silberschmidt 395

Fusarium wilt of garden stock (Mathiola incana)
Kenneth F. Baker 399

Field control of blind seed disease of perennial ryegrass in Oregon
John R. Hardison 404

Book Reviews 420

PHYTOPATHOLOGY

An International Journal
Official Organ of The American Phytopathological Society

EDITORS

HELEN HART, Editor-in-Chief University Farm, St. Paul 1, Minnesota.
W. H. BURKHOLDER, Cornell University, Ithaca, New York.
JAMES H. JENSEN, North Carolina State College, Raleigh, North Carolina.
H. A. RODENHISER, United States Horticultural Station, Beltsville, Maryland.

ASSOCIATE EDITORS

G. M. ARMSTRONG, Clemson Agricultural College, Clemson, South Carolina.
DONALD E. BLISS, Citrus Experiment Station, Riverside, California.
DONALD CATION, Michigan State College, East Lansing, Michigan.
F. L. DRAYTON, Central Experimental Farm, Ottawa, Canada.
A. L. HARRISON, Tomato Disease Laboratory, Yoakum, Texas.
J. G. HARRAR, Calle Viena 26, Mexico D. F., Mexico.
RAY R. HIRT, New York State College of Forestry, Syracuse 10, New York.
BYRON R. HOUSTON, University of California, Davis, California.
M. F. KERNKAMP, University Farm, St. Paul 1, Minnesota.
K. W. KREITLOW, U. S. Regional Pasture Research Laboratory, State College, Pennsylvania.
J. D. WILSON, Ohio Agricultural Experiment Station, Wooster, Ohio.

BUSINESS MANAGEMENT

M. C. RICHARDS, Business Manager, University of New Hampshire, Durham, New Hampshire.
L. J. ALEXANDER, Advertising Manager, Ohio Agricultural Experiment Station, Wooster, Ohio.

NOTICES

All communications regarding subscriptions and back numbers should be sent to M. C. RICHARDS, Business Manager, University of New Hampshire, Durham, New Hampshire.

Subscription Price. $7.50 per year, United States and dependencies; Canada, $7.75; other countries, $8.00; single numbers of the current volume, 75 cents each. The Journal has been issued monthly beginning with Volume 8, January, 1918. Prior to that it was issued bimonthly.

Separate Numbers may be purchased from broken volumes, and stocks available are as follows: Vol. 1, No. 1, index; Vol. 2, Nos. 1, 5, index; Vol. 3, No. 5; Vol. 6, index; Vol. 8, Nos. 2, 3, 5, 6, 7, and 9 through 12; Vol. 9, Nos. 2 through 12, index; Vol. 10, Nos. 2 through 12. Separate numbers of volumes 1 through 10 are sold at $2.00 per copy, those of volumes 11 through 36 at $1.25 a copy. Complete unbound volumes 11 through 36 are available at $10.00 a volume.

Thirty-Year Index (to Vols. 1–30, 1911–1940) bound. $6.25, United States and Canada. $7.50, elsewhere.

Advertisements. Rates may be secured from the Advertising Manager. No indorsement of any statement of claims made in advertisement is assumed by this journal or by The American Phytopathological Society.

Lost Numbers. Claims for copies (domestic subscriptions) lost in the mails must be received within 60 days from the date of issue (Jan. 15 and monthly thereafter); otherwise the usual charge of 75¢ per copy will be made for replacements. Deliveries overseas are not guaranteed and foreign mailings are made at the subscriber's risk.

Separates. No gratis copies supplied. A printed schedule of prices is submitted with the proof and authors may secure separates by placing an order on the blanks provided when proof is returned.

Manuscript may be sent to the nearest member of the editorial board. Clearness, brevity, and conciseness are essential. In form and style, such as punctuation, spelling, and use of italic, manuscript should conform to the best usage in recent numbers of this journal. Manuscripts should be typed on one side of the paper, double spaced, and sent unfolded. Papers longer than four printed pages should include a summary. The name and address of the institution at which the work was done should be included at the end of the summary. Footnotes should be numbered consecutively with arabic numerals except in tables, where small letters should be used. Each table should have a heading stating clearly and concisely its contents. References to literature should be made as footnotes only when five or fewer citations are given. The simplified method of citing literature used in recent numbers of this journal should be followed. References in "Literature Cited" should be listed alphabetically and numbered consecutively. Authors are advised to read "The preparation of manuscripts for Phytopathology" by A. J. Riker, published in Phytopath. 36: 953–977. 1946.

Illustrations necessarily must be limited in number. Photographs, to reproduce satisfactorily, must be of best quality. Authors who desire unusual numbers of illustrations or special types of illustrations will be asked to bear part of the cost, i.e. in excess of $12.50, the approximate cost of 1½ full-page cuts. All illustrations are reproduced on the same grade of paper and will be run as text figures designated by arabic numerals. Individual illustrations within a text figure should be designated by letters. Full-page figures should be arranged to conform to journal page size and shape (4½ × 7 inches) after making due allowance for reductions. Specific reference to each illustration must be made in the text. Each illustration must be accompanied by an adequate legend, the legend to be typed on separate paper and not on the illustration.

The responsibility for statements, whether of fact or opinion, rests entirely with the writers thereof.

The American Phytopathological Society

FOUNDED 1909. INCORPORATED 1915

The A. P. S. Council for 1948

President—R. S. KIRBY, Pennsylvania State College, State College, Pennsylvania.
Vice-President—W. D. VALLEAU, Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.
Secretary—CURTIS MAY, Plant Industry Station, Beltsville, Maryland.
Treasurer—M. C. RICHARDS, University of New Hampshire, Durham, New Hampshire.
Editor-in-Chief—HELEN HART, University Farm, St. Paul 1, Minnesota.

Councilors:

PAUL R. MILLER, Plant Industry Station, Beltsville, Maryland.
S. G. LEHMAN, North Carolina State College, Raleigh, North Carolina.
S. A. E. MCCALLAN, Boyce Thompson Institute for Plant Research, 1086 North Broadway, Yonkers 3, N. Y.
JAMES H. JENSEN, North Carolina State College, Raleigh, North Carolina.
MAX W. GARDNER, University of California, Berkeley, California.
S. J. P. CHILTON, Louisiana State University, University, Louisiana.
A. J. RIKER, University of Wisconsin, Madison 6, Wisconsin.

Sustaining Associates:

B. F. GOODRICH CHEMICAL COMPANY, Rose Building, Cleveland 15, Ohio.
MALLINCKRODT CHEMICAL WORKS, Second and Mallinckrodt Street, St. Louis 7, Missouri.
SINDAR CORPORATION, 330 West 42nd Street, New York 18, N. Y.
ROHM and HAAS, Washington Square, Philadelphia 5, Pennsylvania.
E. I DU PONT DE NEMOURS & COMPANY, Wilmington 98, Delaware.
FREEPORT SULPHUR COMPANY, New Orleans 5, Louisiana.
TENNESSEE CORPORATION, 619–27 Grant Building, Atlanta 1, Georgia.
PILLSBURY MILLS, INC., Metropolitan Building, Minneapolis, Minnesota.
ROGERS BROS. SEED CO., INC., Idaho Falls, Idaho.
TRIARCH BOTANICAL PRODUCTS, Ripon, Wisconsin.
YODER BROTHERS, INC., Barberton, Ohio.
BUTLER COUNTY MUSHROOM FARM, INC., West Winfield, Pennsylvania.
PABST BREWING COMPANY, Milwaukee, Wisconsin.
ASSOCIATED SEED GROWERS, INC., New Haven 2, Connecticut.
FERRY-MORSE SEED CO., Detroit, Michigan.
THE DOW CHEMICAL COMPANY, Midland, Michigan.
LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, Pearl River, N. Y.
NORTHRUP, KING & COMPANY, Minneapolis 13, Minnesota.

FUSARIUM DISEASES OF BROAD BEAN. II. FURTHER STUDIES ON BROAD BEAN WILT CAUSED BY FUSARIUM AVENACEUM VAR. FABAE[1]

T. F. Yu and C. T. Fang[2]

(Accepted for publication December 15, 1947)

In an earlier paper (29) on wilt disease of broad bean caused by *Fusarium avenaceum* var. *fabae*, the symptoms of the disease and the morphological and cultural characters of the causal fungus were described. Since then investigation on the physiology, mode of infection and life history of the fungus, pathologic histology, and factors influencing the development of the disease were made and the results are herein presented.

THE FUNGUS

Isolates of *Fusarium avenaceum* obtained from the diseased roots of broad bean, like those occurring on other crop plants (27), comprise physiological races which are morphologically identical but pathogenically different. Some of these races cause only foot-rot, others cause typical wilt, and still others may cause either a foot-rot or sometimes wilt, dependent largely on environmental conditions. The results of a comparative study on the morphology, physiology, and pathogenicity of these races will be presented in a later report. In the present investigation, isolate No. 607–R–1, a typical wilt producing strain, was used throughout.

PHYSIOLOGY OF THE FUNGUS

Temperature in relation to the growth of the fungus. The relation of temperature to the growth of *Fusarium avenaceum* var. *fabae* was studied in Petri plate cultures of potato-dextrose agar which had been adjusted to pH 7. Four inoculated plates were placed in controlled incubators at each of the following temperatures: 20°, 22°, 24°, 26°, 28°, and 30° C. After the colonies were established the radial growth in each plate was recorded every 24 hours until the thallus covered the entire plate. The average daily growth was plotted for each of the other five temperatures (Fig. 1).

The fungus grew most rapidly at temperatures between 22° and 26° C., with the optimum at 24°–26° C. At 20° and 28° C., the rate of growth was greatly decreased. In another experiment, the fungus was incubated at both higher and lower temperatures. Almost no growth was detected at the higher temperatures tested (33°–35° C.) and very little at the lowest (5°–6° C.).

Hydrogen-ion concentration in relation to the growth of the fungus. Potato-dextrose agar was adjusted by the colorimetric method to the fol-

[1] Paper No. 33 from Division of Plant Pathology, Institute of Agricultural Research, National Tsing Hua University, China.

[2] Division of Plant Pathology, Institute of Agricultural Research, National Tsing Hua University.

lowing hydrogen-ion concentrations: 4.4, 5.2, 5.8, 6.2, 6.7, 7.1, 7.6, and 8.1. Transfers of the fungus were made to the centers of a series of Petri dishes each containing 15 cc. of the medium and the plates, in triplicate, were incubated at 26° C. The diameters of the colonies were measured every 24 hours. The results of the experiment, presented graphically in figure 2,

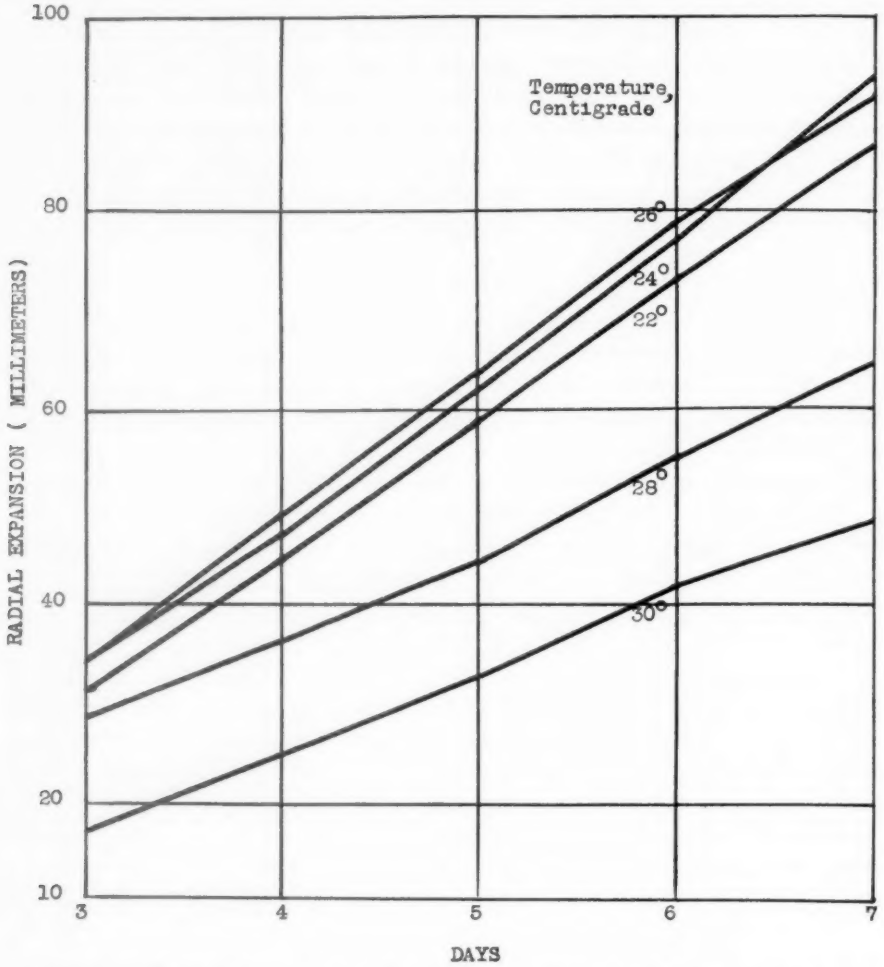


FIG. 1. Daily radial expansion of *Fusarium avenaceum* var. *fabae* on potato-dextrose agar plates incubated at various temperatures.

indicate that the fungus grew in a wide range of pH concentrations, from 4.4 to 8.9 with the optimum between pH 5.8 and pH 6.7. The fungus grew vigorously on the acid media and the growth rate fell rapidly when media of greater alkalinity were used. The fungus tends to increase the acidity of a medium on which it grows.

Spore germination. In moist Petri dishes containing hanging drops of spore suspension, spores germinated most rapidly at temperatures near

the optimum for growth of the mycelium. At those temperatures almost 100 per cent of the spores germinated after 48 hours; while at 10° C. only 30 per cent or less had germinated. At temperatures above 30° C. the percentage of germination decreased. Spores germinated equally well in light or in darkness.

Sporulation. The relation of temperature to the frequency and abundance of sporulation of the fungus was determined both macroscopically and microscopically on agar slants incubated at various temperatures. The results of the test indicate that pionnotes and sporodochia were produced abundantly at temperatures from 10° to 28° C. with the optimum

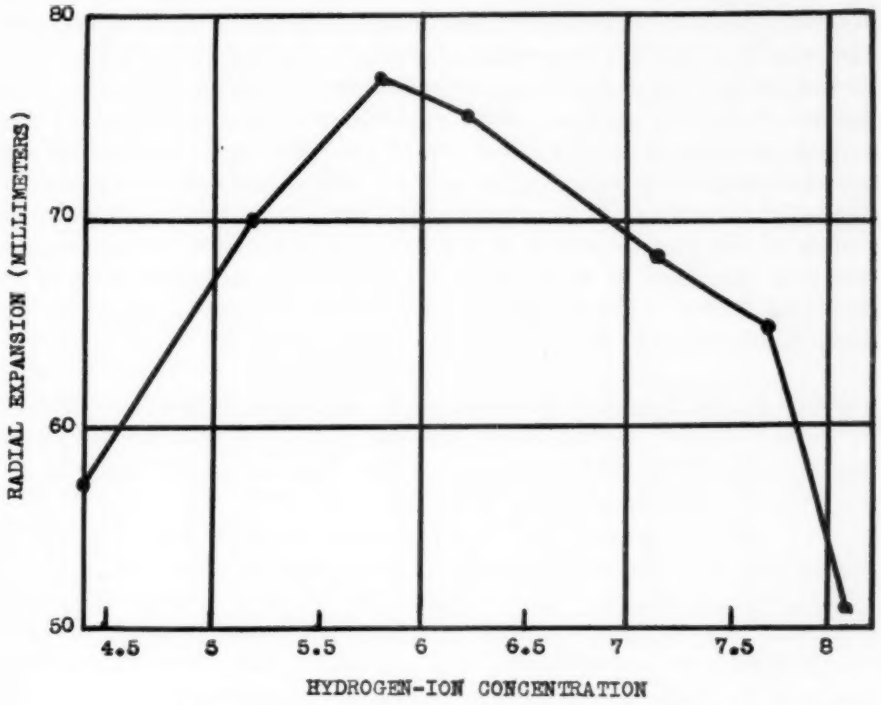


FIG. 2. Average diameters of 8-day-old plate cultures of *Fusarium avenaceum* var. *fabae* on potato-dextrose agar of various hydrogen-ion concentrations.

near 20° C. When spores were transferred to agar slants kept at 18°–20° C., abundant sporulation with slight mycelial growth appeared from 5 to 7 days after the transfer. In general, temperatures slightly lower than those for optimum growth of mycelium were favorable for sporulation.

ENVIRONMENTAL FACTORS IN RELATION TO THE DEVELOPMENT OF THE DISEASE

Soil moisture. Soil moisture is one of the important factors that may influence the development of soil-borne diseases. Linford (17) found that soil moisture had less influence than soil temperature upon pea wilt caused

by *Fusarium oxysporum*. Nevertheless, he found that wet soil slightly favored early development of disease symptoms but drier soil induced more rapid death of affected plants. Starr (21) reported that Alaska pea developed more wilt with an increase of soil moisture. According to Virgin and Walker (28), there was little difference in the rate of wilting caused by the near-wilt organism in dry and medium-moist soils when susceptible varieties of peas were tested. On the other hand, wilting was most rapid in medium-moist soil if resistant varieties were used.

Soil moisture studies were conducted in the greenhouse at an air temperature of 20°–26° C. Twelve-inch flower pots, coated and sealed at the bottom with paraffin, were filled with equal amounts of air-dried soils, the water-holding capacities of which had been determined previously. The soils were arbitrarily designated as dry, medium-moist, and wet, according to their moisture content. Surface-disinfected seed was planted at the rate of 5 seeds per pot. Each day the pots were brought up to the desired moisture content by first weighing them and then adding the necessary amount of water. For the first 7 days, heavy spore suspensions instead of pure water were added to the soils. In addition to these three groups of soil, certain pots of soil were inoculated with spore suspension, then kept saturated by flooding water over the soil surface to a depth of about 1.5 inches. Percentages of water-holding capacity for each soil group and percentages of wilted plants are given in table 1.

TABLE 1.—*The relation of soil moisture to the development of wilt in broad bean*

Condition of soil[a]	Water-holding capacity of soil in per cent	Total no. of plants	No. of wilted plants	Percentage of wilted plants
Dry	18–27	94	68	72.3
Medium-moist	44–65	91	71	78.0
Wet	70–89	95	12	12.6
Saturated	100	96	3	3.1

[a] Each group consisted of 10 pots.

The percentages of wilted plants in the wet and saturated soils were much lower than those in the other two soil groups (Table 1). The percentage of wilted plants in medium-moist soil was slightly higher than that in dry soil. This difference, however, was very slight and statistically insignificant. It was noted that plants growing in dry soil were so poor and stunted it was sometimes difficult to distinguish a wilt from the natural yellowing and drying that often occur with drought. The fact that the lowest percentage of wilted plants occurred in saturated soil confirmed results of the field surveys showing that there was less wilt in low fields where the soil remained saturated throughout the year. The reason for this is still unknown. It is probable that the vigorous growth of the plants in saturated soil may increase the disease tolerance of broad beans. The

results of the experiment, which was not an extensive one, show that broad bean developed less wilt with an increase of soil moisture.

Soil temperature. Soil temperature is by far the most important factor limiting the development and severity of wilt disease caused by species of *Fusarium* in crop plants such as cabbage (9, 24, 26), flax (14, 25), bean (2), tomato (4, 5), tobacco (12), pea (13, 28), potato (10), and watermelon (18, 19, 23).

Experiments for determining the soil temperature in relation to the development of wilt in broad bean have not been made. Field surveys, however, indicated that soil temperature is an important factor in limiting the distribution of the disease. Wilt, although occurring throughout the bean growing sections, was more destructive and prevalent in the northern part of Yunnan Province. In the southern part, where soil temperature was high, the disease was never destructive. The soil temperatures of eastern and western parts which were in the same latitude usually varied but little and the prevalence of the disease seldom varied. It seemed, therefore, that the distribution of wilt disease of broad bean in Yunnan was largely due to a difference of soil temperature.

Soil fertility. The application of chemicals and fertilizers to the soil may at times lessen the effect of certain root diseases although this procedure has seldom been used successfully on an extensive scale. Hoffer and Carr (11) have shown the pronounced influence of soil fertility on the development of root-rot of corn. Linford (17) stated that soil naturally rich in organic matter appeared more favorable for the development of pea wilt than poorer soils.

Although field surveys did not indicate any significant relation between soil type and the prevalence of wilt, it seemed that poor soils were more favorable for severe development of the disease than fertile soils. Experiments, therefore, were conducted to determine the influence of fertilizer on the development of wilt in broad beans. The tests were made on a very poor soil, using ammonium sulfate at the rates of 40, 60, and 120 pounds per Chinese mow[3]. Each plot consisted of 5 rows, 24 feet long and 2.5 feet apart. Seventy-five surface-disinfected seeds were sown in each row. The plots were replicated four times and arranged at random. Pure cultures of the fungus grown on sterilized barley seed were inoculated into the soil at the time of planting. The last records were taken at the end of February when the average percentages of wilted plants of the three treatments were respectively 62, 65, and 29, with the lowest readings occurring on the plots receiving the largest quantity of fertilizer. No further record was made after this time because of the appearance of foot-rot in most of the plots. The difference in percentages of wilted plants between plots to which 40 and 60 pounds of fertilizers per mow had been applied was statistically not significant. The plots receiving 120 pounds of fertilizer per mow produced the lowest percentage of wilted plants and the most vigorous

[3] A Chinese mow is equivalent to 1/6 acre.

plants. This again indicated that the vigorous growth of plants may possibly delay the development of the disease.

Soil reactions. Starr (21) found that the greatest number of pea plants wilted by *Fusarium oxysporum* appeared in soils with pH of 8.5. Wilting occurred over the entire alkaline range and none was found at pH 5.07 or less.

In preparation of soils with different pH values, sulfuric acid and sodium hydroxide were used in varying quantities and thoroughly mixed with soil consisting of one part sandy loam to three parts clay. The soil was allowed to stand for 10 days, in plots which had been previously sealed at the bottom with paraffin, and it was watered plentifully in the meantime. At the end of this period, the hydrogen-ion concentration of the soils was tested with a potentiometer. After the seed was sown, heavy spore suspensions were inoculated into the soil at the rate of 200 cc. per pot. The results of this experiment are given in table 2.

TABLE 2.—*Effect of hydrogen-ion concentration of the soil upon the development of wilt in broad bean*

pH value of soil	Total no. of plants	No. of diseased plants	Percentage of diseased plants
5.02–5.44	93	19	20.4
6.25–6.67	138	79	57.2
6.83–7.09	174	59	33.9
7.76–8.12	101	44	43.5
8.52–8.87	78	11	14.1

The difference in the total number of plants for each pH range was due to the varying number of pots and not to the effect of soil reactions on emergence of seedlings. Bean seed germinated and plants grew vigorously over the entire pH range. Abundant wilted plants appeared in the interval between pH 6.25 to pH 7.09, with the largest number occurring between pH 6.25 to pH 6.67. The results indicate that soil with slight acidity is most favorable to the development of the disease. Since wilting occurred over the entire range of pH tested, the application of fertilizers to change the soil reaction would not be of much value.

Surveys of soil reaction were made on high and low bean lands heavily infested with the wilt organism in Kunming, China, and its vicinity. The soil reactions of 14 fields on high land were all slightly acid. The soils of 9 out of 22 fields on low land had either neutral or slightly alkaline reaction, and those of the remainder were slightly acid. It seems, therefore, that hydrogen-ion concentration of soil is not an important factor in limiting the development of wilt disease.

PATHOGENICITY

In a previous paper (29), it was reported that the fungus was unable to cause infection of common bean, cowpea, tomato, potato, wheat, corn,

oats, and carrot. Further inoculation experiments were made from time to time with attempts to find other hosts of the organism. Negative results were obtained with the following plants, including several legumes: rice, sweet potato, pepper, radish, soybean, hairy vetch, white sweet clover, white clover, and red clover. It was found that the fungus occasionally attacks pea and common vetches and causes yellowing in the lower leaves.

MODE OF INFECTION AND PATHOLOGIC HISTOLOGY

Infection may occur through rootlets, main roots, and hypocotyl. In the field, infection occurs chiefly through the rootlets. The first symptom of the diseased root is the discoloration and shrivelling of rootlets: these frequently yield cultures of *Fusarium avenaceum*, many of which are of the typical wilt producing strain. Infection appears to occur on any part of the main root although it takes a long time for the fungus to reach the inner part of the root through the epidermis (Fig. 3, A). The organism is different from the typical foot-rotting strains which usually produce conspicuous cortical lesions and more rapid collapse of cortical tissues.

Sometimes lesions are found on hypocotyls of young seedlings. In order to demonstrate that the fungus may infect the plants through hypocotyls, the following test was made. Surface-disinfected seed was planted in flower pots containing steam-sterilized soil. After the seedlings attained a height of about 3 inches, a layer of soil was removed and a mixture of melted paraffin and beeswax poured on the newly-exposed surface. A layer of infested soil was then laid on the paraffin layer which placed the diseased soil in direct contact with the hypocotyl. Seedlings were dug out at regular intervals for laboratory examination. Isolations made from the disease lesions on the hypocotyl yielded typical cultures of the fungus.

Microscopic examination of an infected root indicated that the most significant development of the fungus occurred in the vascular tissues, especially the lignified xylem vessels (Fig. 3, B). The fungus rapidly grew upward along the tracheae to the stem. In the meantime, it penetrated the thick-walled xylem and invaded other vascular tissues. Certain xylem vessels contained granular gumlike deposits (Fig. 3, B, C, and D). After the plant had completely wilted, mycelia of the fungus were found in the cortex region. The distribution of the fungus in the stem tissues was the same as that in the root. Abundant mycelia were first found in the lignified xylem vessels (Fig. 3, C). The fungus then penetrated the xylem wall to the cortex and from there invaded cambium and phloem (Fig. 3, D). Only in the latest development of the disease were mycelia found sparingly in tissues outside the endodermis. The fungus always grew upward and, in the later stage of the disease, usually extended through two-thirds of the stem. In no instance, however, could the fungus be isolated from the flowering pedicels.

LONGEVITY IN SOIL

Wilt producing species of *Fusarium* can usually live in soil for a considerable time. Starr (21) reported pea wilt was very destructive in a

field in southern Minnesota that had not grown a crop of peas for the previous six years. Porter (18) found that *Fusarium niveum* can live for at least 16 years in the soil in the absence of watermelon plants.

Diseased roots of broad bean were buried below the soil surface in 12-inch flower pots partly sunken in the ground outside the laboratory. At

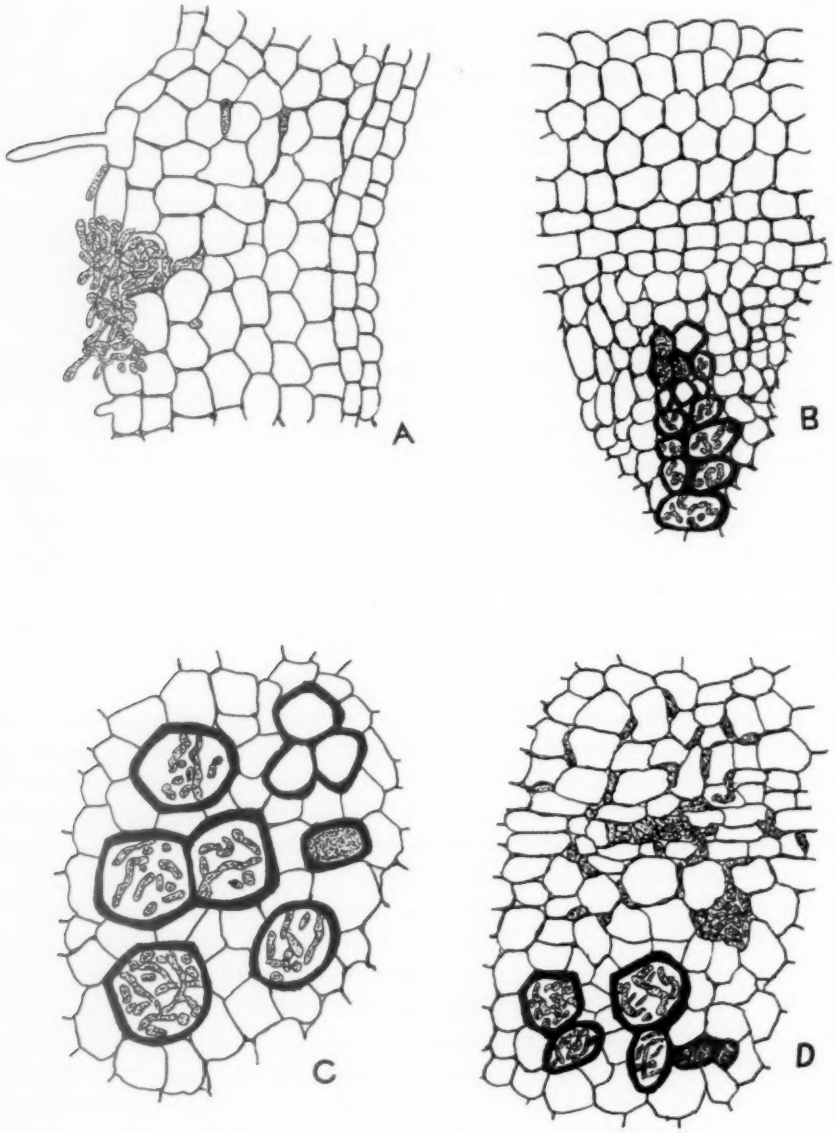


FIG. 3. Cross-sections of young root and stem of a diseased broad bean plant infected by *Fusarium avenaceum* var. *fabae*. A. Invasion of cortical region of the root by the fungus; B. Mycelium of the fungus in the xylem vessels of a root; C. Mycelium of the fungus in lignified xylem of the stem; and D. Invasion of lignified xylem, unlignified xylem, cambium, and phloem of the stem.

regular intervals, pots were removed to the greenhouse and planted with beans. The fungus remained viable for at least three years under these experimental conditions as indicated by the production of wilted plants.

OVERWINTERING STUDIES

Fungi causing root-rot and wilt frequently overwinter on old stubble and other debris in the soil. The experiment on the longevity in soil of *Fusarium avenaceum* var. *fabae* described above indicates that the fungus may live in the diseased roots throughout the winter. When cultures of the fungus grown on sterile wheat kernels were added to soil in flower pots and left outdoors, the fungus frequently sporulated in the spring on certain wheat kernels exposed on the soil surface. When these pots of infested soil were planted with broad bean seed, wilted plants were produced, showing that the fungus readily overwinters on debris in the soil or on its surface.

DISSEMINATION

Most of the wilt diseases caused by species of *Fusarium* have been found to be transmitted by seeds. Bolley (1) reported that flax wilt can be spread by way of seed on which spores of the fungus were lodged with dust at the time of threshing. Fulton and Winston (8) and Porter (18) have demonstrated the spread of watermelon wilt by diseased seeds. According to Edgerton and Moreland (5), spores of *Fusarium lycopersici* will remain on the surface of tomato seeds for about three months. Elliot and Crawford (7) showed that tomato seed naturally infected with *F. lycopersici* will carry the disease from field to field. The cotton-wilt fungus was also found to be seed-borne (6). In the case of pea wilt, Snyder (20) reported that it is sometimes transmitted by way of seed. Starr (21) found over 26 per cent of isolations from the seed coat of peas yielded the wilt organism, while no culture was obtained from cotyledons. Kadow and Jones (15) reported pea wilt fungus was carried to some extent by seed. On the other hand, Stokdyk (22) reported unsuccessful attempts to isolate *F. conglutinans* from cabbage seed and Kendrick (16) was unable to find the wilt organism *F. tracheiphilum* in cowpea seed. Linford also found no evidence of the wilt fungus being carried in pea seed.

In order to learn whether the broad bean wilt organism is seed-carried, pods were collected from wilted plants whose disease was induced by artificial inoculations of the plants with pure cultures of the fungus. The identity of the disease was confirmed by isolation experiments. Seeds were removed from the pods aseptically and stored in glass bottles. Isolations made from the seed failed to demonstrate the presence of the fungus either on the seed coat or in the cotyledons. On the other hand, it is interesting to note that, in another course of studies of the fungus flora of broad bean seed, cultures of *Fusarium avenaceum* var. *fabae* were obtained from the seed coat, but that isolations made from cotyledons failed to yield cultures of the fungus. Apparently, the fungus may be present on the coat of broad

bean seed as a result of contamination at the time of threshing. In harvesting, the entire bean plants including root systems are pulled out and carried to the farm yard where they are beaten by means of a swing-rod until all seeds are shed from the pods. Since *F. avenaceum* var. *fabae* sporulates freely on the basal stem of the diseased plant, this method of threshing offers ample opportunity for the seed to become contaminated with the spores of the fungus.

In Yunnan Province, broad bean is a winter crop and is rotated with rice. After the harvesting of beans in the first part of May, the rice fields are plowed and flooded in time for the transplanting of rice seedlings. As soon as the rice approaches maturity, the water is drained off and broad bean is sown between the rice plants. Since a rice field is flooded for about 4 to 5 months during the year, this led to investigating the question of how long the fungus would remain alive when submerged in water. Sterile barley seed on which the fungus had been grown was mixed with steam-sterilized soil in large Erlenmeyer flasks. These were incubated at 26° C. for one month, then flooded with a layer of sterile distilled water over the soil surface. At regular intervals, a portion of the soil was removed from the flask and placed on a potato-dextrose-agar plate. The results of this test showed that the fungus could live submerged for at least 12 months. It will be seen, therefore, that water drained from a rice field which has been infested with the wilt organism may serve as an important means of spreading the disease.

SUMMARY

1. *Fusarium avenaceum* comprises physiologic races which differ in pathogenicity by producing either foot-rot or wilt of broad bean. In this paper, results of investigation of a typical wilt producing strain designated as *F. avenaceum* var. *fabae* are reported.

2. Growth-temperature studies of the organism in Petri-dish cultures were made. The optimum for mycelial growth was 24°–26° C., the maximum below 33° C., and the minumum 5°–6° C.

3. The organism was capable of growing in media having a range of hydrogen-ion concentration from pH 4.5 to pH 8.9. Growth was most rapid between pH 5.8 and pH 6.7.

4. Spores germinated over a wide range of temperature and most rapidly when temperatures approached the optimum for mycelial growth. Temperatures around 20° C. were most favorable for sporulation of the fungus.

5. Dry and medium-moist soils were better for the development of the disease than wet and saturated soils. Medium-wet soil favored early development of symptoms; and dry soil, rapid death of affected plants.

6. No experiments were made to determine the influence of soil temperature upon broad bean wilt, although field surveys indicate that it is an important factor in limiting the distribution and severity of the disease.

7. The influence of soil reaction on wilt development was tested by growing broad bean plants in infested soils with a hydrogen-ion concentra-

tion varying from pH 5.02 to pH 8.87. The largest number of wilted plants occurred over a range of pH 6.25 to pH 6.67, although wilting might occur at any point over the entire range.

8. Fertilizers were found to have some influence on the development of wilt disease. More wilted plants were found in soils that had received the least quantity of ammonium sulfate. Poorer soils were more favorable than richer soils for the development of the disease.

9. Infection of the plant may occur through rootlet, main root, and hypocotyl. Of the three, rootlet infection is by far the most important in the field. The fungus invades chiefly the xylem of the root and stem, and from xylem spreads to cambium and phloem tissues. In the cortex, the fungus grew slowly and sparingly and it was only in the late stage of the disease that the fungus was found in the cortex of a diseased bean stem.

10. This fungus occasionally attacks peas and common vetches.

11. The fungus remained viable for at least three years in diseased roots buried under the soil surface. In the field it lives for a considerable time on old stubble and other debris in the soil.

12. No evidence has been found that the fungus was present inside the seed; but it was found on the seed coat, possibly by contamination at threshing time.

NATIONAL TSING HUA UNIVERSITY,
PEIPING, CHINA

LITERATURE CITED

1. BOLLEY, H. L. Flax and flaxseed selection. N. Dak. Agr. Exp. Sta. Bul. 55. 1903.
2. BURKHOLDER, W. H. The dry root-rot of the bean. Cornell Univ. Agr. Exp. Sta. Mem. 26. 1919.
3. ———. Effect of the hydrogen-ion concentration of the soil on the growth of the bean and its susceptibility to dry root-rot. Jour. Agr. Res. [U.S.] 44: 175–181. 1932.
4. CLAYTON, E. E. The relation of temperature to the fusarium wilt of the tomato. Amer. Jour. Bot. 10: 71–88. 1923.
5. EDGERTON, C. W., and C. C. MORELAND. Tomato wilt. La. Agr. Exp. Sta. Bul. 174. 1920.
6. ELLIOT, J. A. Cotton wilt, a seed-borne disease. Phytopath. 12: 50–51. 1922.
7. ———, and R. F. CRAWFORD. The spread of tomato wilt by infected seed. Phytopath. 12: 428–434. 1922.
8. FULTON, H. R., and J. R. WINSTON. Watermelon wilt spread by contaminated seed. Bien. Rept. N. Car. Agr. Exp. Sta. 1913–1914: 48–51. 1914.
9. GILMAN, J. C. Cabbage yellows and the relation of temperature to its occurrence. Ann. Mo. Bot. Gard. 3: 25–84. 1916.
10. GOSS, R. W. Potato wilt and stem-end rot caused by Fusarium eumartii. Neb. Agr. Exp. Sta. Res. Bul. 27. 1924.
11. HOFFER, G. N., and R. H. CARR. Accumulation of aluminum and iron compounds in corn plants and its probable relation to rootrots. Jour. Agr. Res. [U.S.] 23: 801–823. 1923.
12. JOHNSON, J. Fusarium-wilt of tobacco. Jour. Agr. Res. [U.S.] 20: 515–535. 1921.
13. JONES, F. R. Stem and rootrot of peas in the United States caused by species of Fusarium. Jour. Agr. Res. [U.S.] 26: 459–475. 1923.
14. JONES, L. R., and W. B. TISDALE. The influence of soil temperature upon the development of flax wilt. Phytopath. 12: 409–413. 1922.
15. KADOW, K. J., and L. K. JONES. Fusarium wilt of peas with special reference to dissemination. Wash. Agr. Exp. Sta. Bul. 272. 1932.
16. KENDRICK, J. B. Seed transmission of cowpea fusarium wilt. Phytopath. 21: 979–983. 1931.
17. LINFORD, M. B. A fusarium wilt of peas in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 85. 1928.

18. PORTER, D. R. Infection studies with watermelon wilt caused by Fusarium niveum EFS. Iowa Agr. Exp. Sta. Res. Bul. 112. 1928.
19. ————, and I. E. MELHUS. The pathogenicity of Fusarium niveum (EFS.) and the development of wilt resistant strains of Citrullus vulgaris (Schrad.) Iowa Agr. Exp. Sta. Res. Bul. 149. 1932.
20. SNYDER, W. C. Seed dissemination in Fusarium wilt of pea. Phytopath. 22: 253–257. 1932.
21. STARR, G. H. A study of diseases of canning crops (peas and corn) in Minnesota. Minn. Agr. Exp. Sta. Tech. Bul. 89. 1932.
22. STOKDYK, E. A. Progress report of cabbage yellows investigation in Kansas. (Abstr.) Phytopath. 14: 24. 1924.
23. TAUBENHAUS, J. J. Wilts of the watermelon and related crops. Tex. Agr. Exp. Sta. Bul. 260. 1920.
24. TIMS, E. C. The influence of soil temperature and soil moisture on the development of the yellows in cabbage seedlings. Jour. Agr. Res. [U.S.] 33: 971-992. 1926.
25. TISDALE, W. H. Relation of temperature to the growth and infecting power of Fusarium lini. Phytopath. 7: 356–360. 1917.
26. TISDALE, W. B. Influence of soil temperature and soil moisture upon the Fusarium disease in cabbage seedlings. Jour. Agr. Res. [U.S.] 24: 55–86. 1923.
27. TU, C. Physiologic specialization in Fusarium spp. causing headblight of small grains. Minn. Agr. Exp. Sta. Bul. 74. 1930.
28. VIRGIN, W. J., and J. C. WALKER. Relation of temperature and moisture to near-wilt of pea. Jour. Agr. Res. [U.S.] 59: 591–600. 1939.
29. YU, T. F. Fusarium diseases of broad bean. I. A wilt of broad bean caused by Fusarium avenaceum var. fabae n. var. Phytopath. 34: 385–393. 1944.

PHOMA ROT OF GARDEN BEETS

BARBARA C. HEIBERG[1] AND G. B. RAMSEY[2]

(Accepted for publication January 7, 1948)

For several years a black rot of topped garden beets in storage has been one of the most important causes of loss of that commodity on the Chicago market during the winter months. Rail shipments received from Canada, New York, and Texas, as well as truck shipments of topped storage beets from Illinois and nearby States, have shown appreciable amounts of black rot on arrival at the wholesale market and in retail stores. Although this disease occurs commonly in topped beets that have been in storage for a few months, it has never been found in young bunch beets on the market. A survey of the literature shows that very little has been published on black rot in garden beets (4, 5, 7), although there is an abundant literature on a similar disease of sugar beets caused by *Phoma betae* (Oud.) Fr. (2, 3, 8). The present investigation was undertaken to determine whether or not black rot in garden beets is due to the same organism and to determine the degree of pathogenicity of the fungus for garden beets.

Storage beets on the market usually show black rot at the tip of the root (Fig. 1, A, B), although it occasionally occurs at the crown and in wounds on the side of the root. Because of the color of the beets, the small lesions are usually overlooked. On casual inspection the decay is seldom observed until the affected tissues become black and slightly sunken or until a flat, grayish white surface mycelium becomes evident. Internally the decayed areas are dark brown to black with a sharp line of demarcation between the healthy and diseased tissue. At first the invaded tissues are brown and water-soaked, but in the older lesions the affected tissues are black and somewhat granular, eventually becoming dry and spongy. With age, cavities lined with mycelium may be found within the spongy tissue of the larger lesions (Fig. 1, B). Although considerable grayish white mycelium develops on the surface of the older lesions under humid conditions no pycnidia have ever been observed on the beets.

Isolations made from the advancing edge of black-rot lesions in storage beets have consistently yielded a species of *Phoma*. Cultures of the organism obtained from garden beets grown in various localities appear to be identical. Furthermore, studies made of five isolates of the garden beet organism in comparison with authentic cultures of *Phoma betae* from sugar beet indicate that they all are the same species. On potato-dextrose agar (pH 7) the garden-beet organism produced abundant pycnidia that ranged from 210 to 560 μ in diameter. The conidia measured 4.3–8.1 $\times$ 2.9–5.8 μ, averaging 5.7 $\times$ 3.5 μ.

[1] Scientific Aide, [2] Senior Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

No significant differences were found in the morphological characteristics of the various isolates from garden beet and sugar beet lesions. The fungus causing black rot of garden beets is therefore considered to be identical with *Phoma betae* (Oud.) Fr., the organism described as the cause of the sugar-beet black rot.

The cultures of *Phoma betae* isolated from garden beets grew on potato-dextrose agar (pH 7) through a temperature range of 35° to 95° F. Optimum growth was obtained at 75° F. The average diameters of colonies developed on potato-dextrose agar in 7 days at temperatures ranging from 35° to 95° F., in 10-degree intervals, are shown in figure 2.

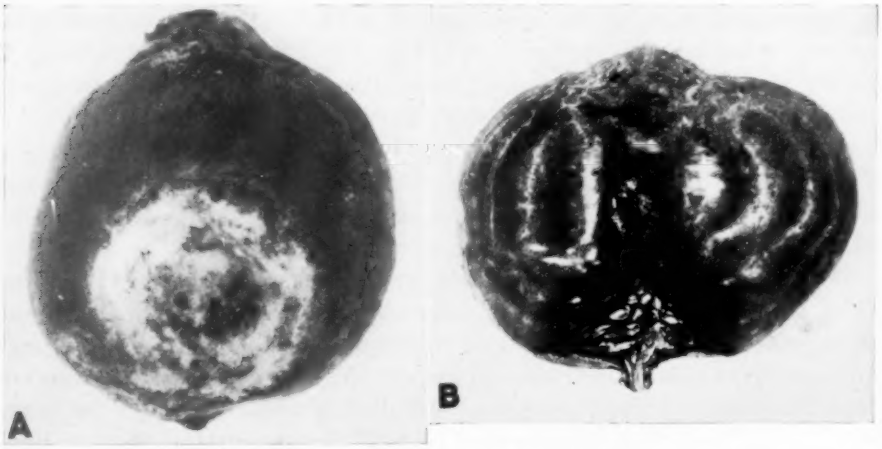


FIG. 1. A. Natural lesion at the tip of the beet, showing surface mycelium. B. Longitudinal section of a beet with natural infection.

In order to establish the degree of pathogenicity of *Phoma betae* for garden beets, both whole and sliced beets were inoculated. In whole beets, V-shape punctures $\frac{1}{8}$ to $\frac{1}{4}$ inch deep were made with a sterile scalpel and a square of agar bearing mycelium and pycnidia was placed in the wound. In other experiments, wells about $\frac{1}{2}$ inch deep were made in whole beets with a sterile $\frac{1}{4}$-inch cork borer and the inoculum was placed in the bottom and the plug replaced.

In preliminary tests it was found that inoculated beets stored in moist sand remained in better general condition and developed more decay than those stored in moist chambers. Beets inoculated by the scalpel method and stored in moist sand for 6 weeks developed lesions averaging 5 mm. in diameter at 45°, 12 mm. at 55°, and 6 mm. at 65° F. Beets inoculated by the well method and stored at 50° for 4 weeks developed internal lesions ranging from 5 to 35 mm. in diameter. Both old storage beets and young bunch beets were inoculated in this fashion and in most instances greater decay developed in the older beets, thus indicating their greater susceptibility to invasion.

In further tests of pathogenicity, disease-free storage beets were cut aseptically into slices ½ inch thick and these were placed on Syracuse dishes in moist chambers with a small amount of water in the bottom of each chamber. Inoculations were made by placing a small amount of agar bearing mycelium and pycnidia from a vigorously growing culture of *Phoma betae* on the center of each slice. The moist chambers were held at 35°, 45°, 55°, 60°, and 65° F. After one month some decay developed throughout this temperature range, but the greatest depth of penetration (10 mm.) occurred at 45° and 55°. At these two temperatures, as well as the other three, the organism grew over the top of the slice to produce a flat gray

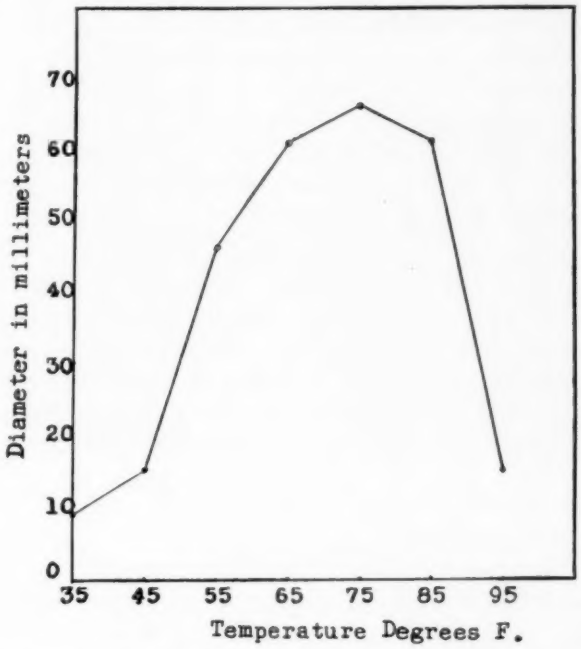


FIG. 2. Growth of *Phoma betae* on potato-dextrose agar (pH 7) in seven days at various temperatures.

mycelium covering ¼ to ¾ of the surface. In other inoculation tests, with slices of old storage beets in comparison with slices of young beets, it was found, as in the previous whole-root inoculations, that the rate of decay was slightly greater in the old beets.

Five different cultures of *Phoma betae* from garden beets and 3 from sugar beets were used in inoculation tests to determine whether there was any difference among them in pathogenicity. Inoculated slices of storage beets were held at 50° F. for one month. At the end of this period all slices showed some decay, but 2 sugar beet isolates of *P. betae* produced slightly more decay than the garden beet isolates. Of the 5 garden beet isolates 2 caused more extensive decay than the other 3, thus indicating the possibility that there may be some difference in pathogenicity between strains of *P. betae*. The organisms were recovered in each case by reisolation.

For inoculation studies with seedlings, seed of the Detroit Dark Red variety was planted in the greenhouse. When the seedlings had developed their foliage leaves they were transplanted to larger pots, three seedlings per pot. When the plants were about one month old two pots were sprayed with a water suspension of *Phoma* spores of a garden-beet isolate, one pot was covered for two days and the other was left uncovered. No spots were ever observed on the leaves of either pot. Two weeks later two more pots were inoculated in the same manner with negative results. One month later two more pots were inoculated; and in this lot, two days after inoculation, lesions were found on the older leaves of the plants in the pot that had been covered, but no infection occurred on the leaves of the plants that had not been covered. A sugar-beet isolate was also used to inoculate a pot of covered plants two months old, and similar leaf spots were produced within two days.

In each case infection occurred only on the older leaves (9 cm. long). The spots produced were small (2 to 3 mm. in diameter) with tan centers and red borders. The lesions did not enlarge much with age. The center of some of the older spots dropped out, producing a shot-hole effect. *Phoma betae* was reisolated from these leaf spots.

Pool and McKay (6) found that only mature sugar-beet leaves are infected with *Phoma betae* and then only through some lesions on the leaf's surface, such as insect injuries. They also found pycnidia developing in 10 days to 2 weeks on lesions 1 to 2 cm. in diameter. In the experiments reported here on garden beets, leaf spots developed on leaves that had not in any known way been injured mechanically. However, the growth of the lesion was halted in some way and no pycnidia developed on lesions one month old.

Repeated attempts to infect beet seedlings by inoculating the soil with water suspensions of spores, by burying the mycelium and pycnidia in the soil, or by burying rotten beets in the soil have failed, except in one case where a piece of inoculum was placed in the hole and a seedling was transplanted on it. These results indicate that the fungus is a weak pathogen and is not able to infect uninjured seedlings. Edson (2) considered *Phoma* capable of infecting beets only during periods of especial susceptibility, such as old age or low vitality.

Pool and McKay (6) list beet balls (seed), wind, irrigation water, insects, and dung as the agents of dissemination of *Phoma* spores in sugar beet fields. They considered the seed to be the most effective agent of dispersal, with wind and irrigation water playing secondary roles. Coons and Stewart (1) found *Phoma* present in carefully selected sugar beet seed.

Seeds of the Detroit Dark Red variety were bought from three commercial sources and germinated in moist cheesecloth. Over half of the seedlings were killed by *Phoma* in two of the lots. In the third lot about 20 per cent of the seedlings were infected. The blackened seedlings were examined microscopically and found to be covered with pycnidia of *Phoma betae*. The

fungus was isolated from the seedlings and was proved to be pathogenic for beets by inoculation tests.

In studies on the longevity of *Phoma betae* in garden-beet seed, Newton and Bosher (5) found that death of seedlings caused by *Phoma* decreased over a period of 5 years from 30 to 5 per cent. No decline in percentage of seed germination was noted.

Edson (2) showed that in sugar beets *Phoma* may live for a long time in inconspicuous lesions on seedlings and roots and eventually cause severe rot of the roots in storage. The studies here presented indicate that infection and decay of garden beets caused by *Phoma betae* probably occurs in the same manner as Phoma rot in sugar beets.

SUMMARY

A black rot of garden beets from storage is described as it occurs under market conditions. Comparison of the fungus isolated from garden beet lesions with *Phoma betae* from sugar beets indicates that they are the same species.

Optimum growth of *Phoma betae* from garden beet on potato-dextrose agar occurred at 75° F. with almost equal growth at 65° and 85°. Wound inoculations produced infection of both old and young garden-beet roots, but most severe decay occurred in the old roots.

Beet plants grown in the greenhouse and atomized, when about 2 months old, with a water suspension of spores of *Phoma betae* developed leaf spots in the older leaves; younger plants similarly inoculated were not infected.

In germination tests in the laboratory, with three lots of commercial garden beet seed, 20 to over 50 per cent of the seedlings were killed by this fungus.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
U. S. DEPARTMENT OF AGRICULTURE,
AND
DEPARTMENT OF BOTANY,
UNIVERSITY OF CHICAGO.

LITERATURE CITED

1. COONS, G. H., and DEWEY STEWART. Prevention of seedling diseases of sugar beets. Phytopath. **17**: 259–296. 1927.
2. EDSON, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. Jour. Agr. Res. [U.S.] **4**: 135–168. 1915.
3. FRANK, A. B. Ueber *Phoma betae*, einen neuen parasitischen Pilz, welcher die Zuckerrüben zerstört. Ztschr. Rübenz. Indus. **42**: 904–916. 1892.
4. HEDGCOCK, G. G. Proof of the identity of Phoma and Phyllostieta on the sugar beet. Jour. Myco. **10**: 2–3. 1904.
5. NEWTON, W., and J. E. BOSHER. The longevity of *Phoma betae* in garden beet seed. Sci. Agr. (Ottawa) **26**: 305–306. 1946.
6. POOL, VENUS W., and M. B. MCKAY. *Phoma betae* on the leaves of the sugar beet. Jour. Agr. Res. [U.S.] **4**: 169–178. 1915.
7. RAMSEY, G. B., and J. S. WIANT. Market diseases of fruits and vegetables: Beets, Endive, Escarole, Globe Artichokes, Lettuce, Rhubarb, Spinach, Swiss Chard and Sweetpotatoes. U. S. Dept. Agr. Misc. Pub. 541. 1944.
8. TOMPKINS, C. M., and S. B. NUCKOLS. The relation of type of topping to storage losses in sugar beets. Phytopath. **20**: 621–635. 1930.

ANGULAR LEAF SPOT OF KUDZU CAUSED BY A NEW SPECIES OF MYCOSPHAERELLA[1]

J. L. Weimer and E. S. Luttrell[2]

(Accepted for publication January 3, 1948)

INTRODUCTION

In October, 1946, a leaf disease of kudzu (*Pueraria thunbergiana* Benth.) not hitherto observed was seen in a planting at Experiment, Georgia. The disease appeared to be responsible for the premature shedding of many leaves and varying degrees of injury to others. Observations were made in other kudzu plantings, and the disease was found at two additional locations, one a 20-year-old planting, also on the Experiment Station grounds, and the other a commercial planting 15 miles distant. In May, 1947, the same disease was found in several counties in central and south Georgia and in two locations in Alabama. Bain and Presley (1) reported having found the same leaf spot in Mississippi in the summer of 1946. The potential seriousness of this disease has made it worthy of further study. The results of an investigation into its nature and cause form the basis of this paper.

SYMPTOMATOLOGY

The disease seems to be limited to the leaves of the kudzu plant, leaves of all ages being susceptible. The lesions usually are angular, although some tend to be circular. Since the angular outline is the most characteristic feature of the lesion, the common name angular leaf spot is suggested (Fig. 1, A).

At first the lesions are evident only on the upper side of the leaf, but eventually they involve the entire thickness of the leaf and are visible on both sides. Young lesions are light brown, but they gradually darken to dark brown or almost black. They often have a yellowish margin or a slight yellowish halo. Old lesions may have dark gray centers with darker margins. The lesions, scattered in a miscellaneous manner over the leaf, are small at first but gradually enlarge, often merge, and eventually involve large areas or even the entire leaflet. In the field individual lesions most commonly vary from 1 to 10 mm. in diameter. The lesions may be more or less thickly covered with dark brown conidial tufts, which are abundant on the upper surface of the leaf, but sparse on the lower surface. When large areas of the leaflet have been killed, as when the lesions are

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 172, Journal Series, Georgia Agricultural Experiment Station.

[2] Senior Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and Associate Botanist, Georgia Agricultural Experiment Station, respectively.

sufficiently numerous or when many of them have coalesced, the remaining tissue may turn yellow and finally brown, and the entire leaflet may fall off. Practically the entire upper surface of some of these dead leaflets may be covered with conidial tufts.

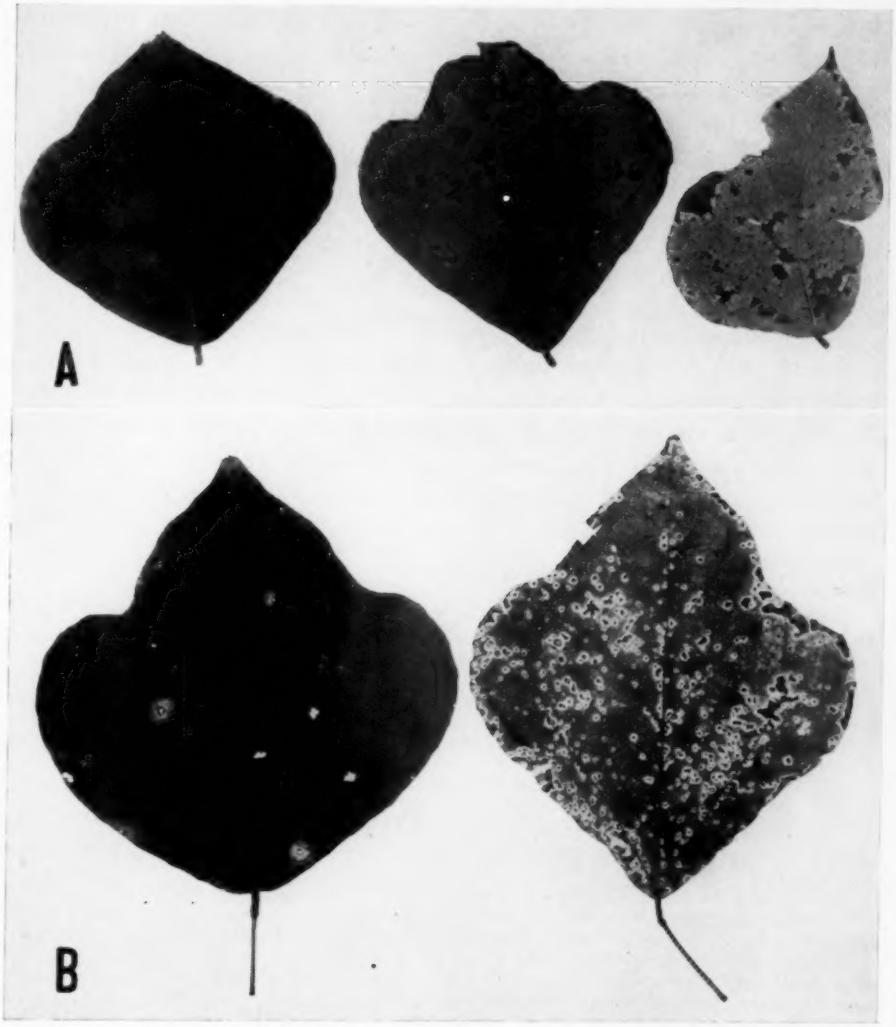


FIG. 1. A. Angular leaf spot on kudzu leaflets resulting from natural infection. Leaflet at the left was healthy. The other two leaflets illustrate the appearance of the lesions on the upper (center) and lower surfaces (right), respectively. $\times \frac{1}{2}$. B. Halo spot on kudzu leaflets resulting from natural infection. The leaflet at the left had lesions with a small necrotic center and large halo and that at the right had halo spot lesions with relatively large necrotic centers and small but very conspicuous halos. $\times \frac{1}{2}$.

So far as known, the only disease of kudzu that might be confused with angular leaf spot is halo spot, (*Pseudomonas phaseolicola* (Burk.) Dowson). In some stages of their development it is difficult to distinguish these two diseases, although in other stages they differ greatly. The halo

spot lesion commonly seen in nature during the early part of the growing season or during wet spells, has a small dead center surrounded by a rather large halo (2, 3). When the spots are numerous the halo is small (Fig. 1, B). When the halo is small and the necrotic center relatively large, halo spot is similar to young lesions of angular leaf spot. In well developed lesions of angular leaf spot, however, the halo is much less pronounced (Fig. 2, A); and if the humidity has been high, conidial tufts of the causal fungus may be seen scattered over the upper surface of the lesion.

Infected leaflets held in a moist chamber over night have bacterial ooze on the lower surface in the case of halo spot or conidial tufts on the upper surface in case of angular leaf spot.

ETIOLOGY

Taxonomy

The fungus associated with angular leaf spot of kudzu appears to be identical with *Cercospora puerariicola* Yamamoto (8). A spermagonial and a perithecial stage have been found in the course of this study. Since the perithecia are spherical, short necked, embedded singly in the host leaf tissue, and have hyalodidymous ascospores and fasciculate, aparaphysate asci, this fungus should be placed in the genus *Mycosphaerella*. Since the perfect stage has not been reported previously, it is described as follows:

Mycosphaerella puerariicola sp. nov.

Stat. Conid.: *Cercospora puerariicola* Yamamoto. Trans. Sapporo Nat. Hist. Soc. 13(3): 142–143. 1934.

Peritheciis plerumque epiphyllis, punctiformibus, in maculis dispositis, nigris, innatis, sphaeroideis, 61–93 μ (medio 71.2 μ) diam., poro praeditis; ascis fasciculatis, aparaphysatis, clavatis, breve stipitatis, octosporis, 44.8–60.8 × 9.3–9.9 μ; ascosporis hyalinis vel pallide viridulis, 1-septatis, ad septum leniter constrictis, fusoideis, rectis vel inaequalibus, utrinque obtusiusculis, distichis, guttulatis, 14.3–22.2 × 2.7–4.5 μ (medio 19.7 × 4.2 μ).

Hab. in vere in foliis dejectis *Puerariae thunbergianae* Benth., Experiment, Georgia, U.S.A.

Spermagoniis nigris, punctiformibus, innatis, globosis, 42–67 μ (medio 52.7 μ) diam., poro praeditis, plerumque epiphyllis, cum stromatibus ascogonialibus in maculis aggregatis; spermatiis hyalinis, bacillaribus, plerumque 3 × 1 μ.

Hab. in autumno in foliis vivis atque dejectis *Puerariae thunbergianae*.

Status conidicus: Maculis 1–10 mm. diam., angulosis, brunneolis, dein nigro-brunneis, zonis flavidis circumdatis; conidiophoris plerumque epiphyllis, simplicibus, 1–6 septatis, non-constrictis, 20–84 × 4–4.5 μ, erectis vel sub-erectis, laxe fasciculatis, olivaceo-brunneis, erumpentibus, e stromate sphaerico, fusco, pseudo-parenchymatico, innato oriundis; conidiis hyalinis vel pallide olivaceis, cylindrico-obclavatis, rectis vel curvulis, 1–16 septatis, non-constrictis, vertice attenuatis, basi rotundatis vel truncatis, 25.2–126 × 3.0–5.3 μ (medio 69.7 × 4.3 μ).

Hab. in vera, aestate, atque autumno in foliis vivis *Puerariae thunbergianae*.

Type collection B.P.I. no. 71516, Experiment, Ga. May, 1947, Weimer and Luttrell.

Collections of both the perithecial and conidial stages have been deposited in the Mycological Collections of the Bureau of Plant Industry, the Farlow Herbarium, Harvard University, and the Royal Botanic Garden at Kew, Surrey, England.

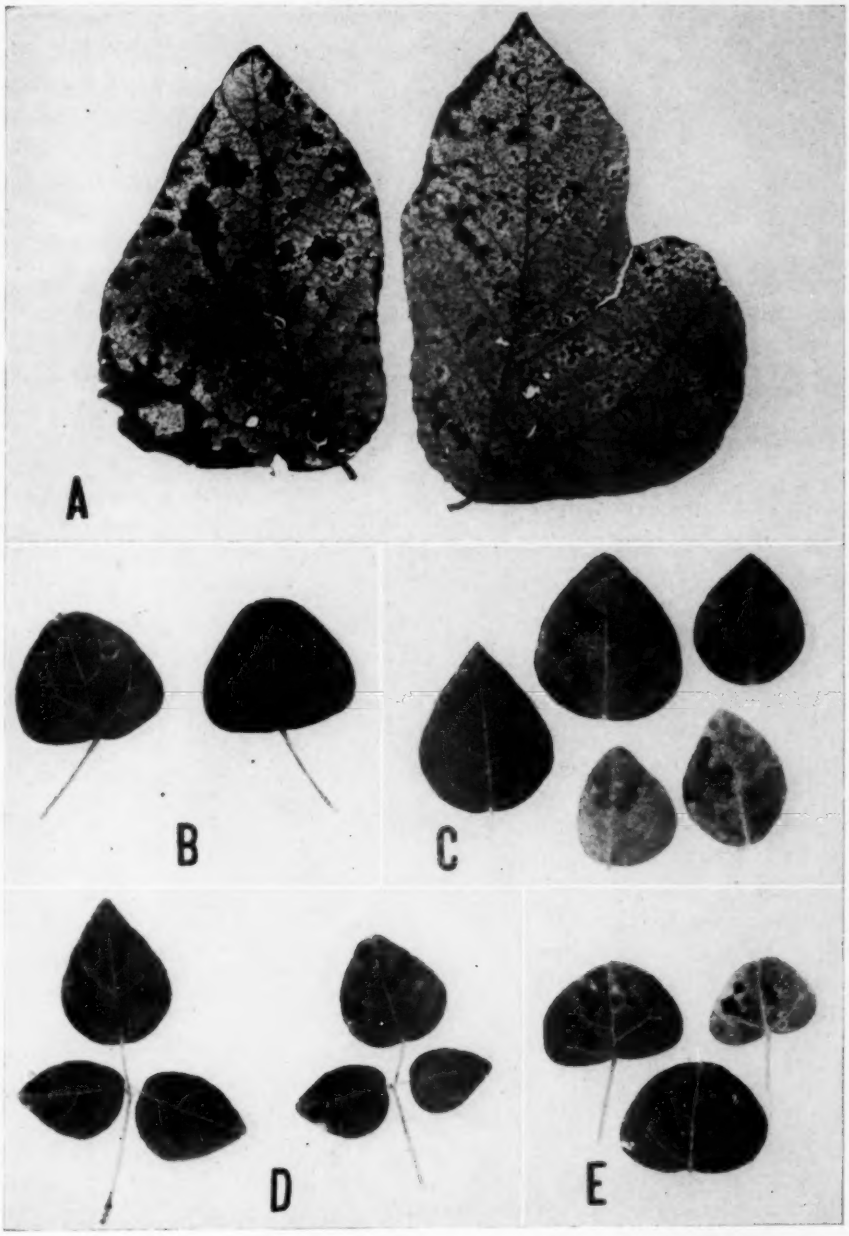


FIG. 2. A. Heavy natural infection of kudzu leaflets with angular leaf spot. Lesions have a relatively large amount of necrotic tissue and small halos. $\times \frac{1}{2}$. B. Two monofoliate kudzu seedling leaves with angular leaf spot lesions resulting from inoculation. Some lesions have a faint or conspicuous yellowish halo but the affected tissue is largely necrotic. Inoculated with a culture of the conidial stage on January 14, 1947, and photographed February 12, 1947. $\times 1\frac{1}{2}$. C. Healthy leaf at left. Upper and lower leaves at right illustrate lesions on the upper and lower surfaces, respectively, 47 days after inoculation in the greenhouse. $\times \frac{3}{4}$. D. Two trifoliate leaves showing angular leaf spot lesions 66 days after inoculation with a conidial culture. $\times \frac{3}{4}$. E. Three monofoliate seedling leaves with lesions resulting from inoculation with a pure line ascospore culture. These lesions are identical with those produced by the conidial culture. $\times 1$.

Isolations and Inoculations

The *Cercospora* stage of the angular leaf spot fungus was isolated by placing infected leaves in a moist chamber for 24 or 48 hours and then transferring spores from the conidial tufts to agar by means of a sterile needle. Tissue plantings from leaves brought from the field often gave a *Colletotrichum* sp. that was acting as a secondary invader. The *Cercospora* grows slowly in culture and produces conidia sparsely on some media. Cultures of the ascospore stage used in these investigations were obtained by catching ascospores shot from pieces of the over-wintered leaves on agar and transferring single spores or groups of spores to agar tubes.

Since conidia from culture were not sufficiently abundant to be satisfactory for inoculation purposes, a combination of macerated hyphae and spores was used. The fungus was grown on a liquid medium made according to Leonian's (5) malt-agar formula with the agar omitted. The fungus colonies were removed from the culture medium, macerated in sterile tap water, and placed on the leaflets to be inoculated.

An inoculation experiment was set up on January 14, 1947, in which very young colonies of the fungus obtained from conidia and grown on Leonian's medium were used as inoculum. The plants inoculated were seedlings grown from seed collected locally, scarified by immersing in commercial sulphuric acid for one hour at 60° F. as described by Tabor (6), planted in sterilized soil in pots, and held in the greenhouse. The plants when inoculated were very small, usually having two monofoliate and one trifoliate leaf each. One or more pieces of inoculum were placed on each monofoliate leaf of 13 plants. Two plants in one pot were held as controls. After being inoculated, the plants were kept in a moist chamber for 96 hours at temperatures varying from 23° to 25° C. No leaf spot resulting from inoculation was positively identified until February 3, 1947, when a few spots from 1 to 2 mm. in diameter were evident on some leaves. These lesions slowly enlarged and additional ones became evident. On February 11, a total of 9 leaves on six inoculated plants had one or more lesions. There was no infection of the control plants. The lesions at first were yellowish to light brown, but gradually darkened to very dark brown, often with a yellowish border as illustrated by two monofoliate leaves shown in figure 2, B. Twenty-four of the 26 leaves inoculated had one or more typical lesions on February 26. The fungus was recovered from some of the infected leaves by placing them in a moist chamber for 24 hours and then picking off spores and transferring them to agar.

A second experiment was initiated on January 27, 1947, using the same methods except that the inoculum was placed on the under sides of the leaves where stomata are much more abundant. Leaves on nine seedlings were inoculated, and two seedlings were held as controls. The temperature in the moist chamber varied from 22° to 27° C. during the time the pots were held there. On February 11, there were no lesions that could

be recognized with certainty, but a few small spots were suspected of being immature lesions. By February 26 there were typical lesions on most of the leaves that had been inoculated. The lesions were sufficiently numerous and large to involve and kill considerable areas of some leaves (Fig. 2, C and D). The fungus was recovered from a number of lesions, and an abundance of spores formed on the lesions when held in a moist chamber for 24 hours. It is evident that in less than a month from the time of inoculation a large part of some leaves had been invaded and killed. For the most part the lesions ranged from 2 to 4 mm. in diameter, except when they had merged and involved larger areas. The earliest symptoms of infection were not apparent until about two weeks after inoculation. Infection can take place from either the upper or the under side of the leaf. Further inoculation experiments demonstrated that the fungus isolated from the lesions resulting from inoculation was equally as pathogenic as the original isolate.

Proof of the pathogenicity of the perfect stage of this fungus was obtained in an experiment initiated April 21, 1947. Young seedlings having only monofoliate leaves and grown from sterilized seed planted in sterilized soil were used. Three cultures were used in this experiment: (1) one of the original conidial cultures whose pathogenicity had been proved, (2) an isolate from a leaf infected in a previous experiment, and (3) a culture from a single ascospore. The inoculated plants were held in the moist chamber at temperatures of 25° to 28° C. for 96 hours. The first evidence of infection was noted on May 7, and by May 14 typical *Cercospora* lesions were present on some of the leaves inoculated with the ascospore culture. On May 19, nine plants inoculated with the ascospore culture had one or more typical lesions on one or both of the leaves inoculated, or a total of 14 out of 18 leaves were infected (Fig. 2, E). Seven of the 10 leaves on five plants inoculated with the orginal conidial isolate and five of the 12 leaves inoculated with the reisolate showed infection. There was no infection on any of the control plants. Thus 100 per cent of the plants inoculated had one or more leaves infected. Perhaps it should be explained that usually only two, rarely three, of the monofoliate leaves are produced by a seedling, hence only two leaves could be inoculated in the earliest seedling stage. The results of this experiment prove the pathogenicity of the ascospore stage and show that it produces lesions identical with those produced by the conidial stage under identical conditions. This constitutes proof of the connection of the conidial and perithecial stages of the fungus.

LIFE HISTORY

Isolates of the fungus grew slowly on all media employed, namely, oat meal, corn meal, Bacto nutrient, and Leonian's malt agars, Leonian's malt agar formula with the agar omitted, sterile oats, and kudzu stems. Of these media, Bacto nutrient agar was the least satisfactory. Leonian's

agar was slightly better than corn meal or oat meal agars. Growth of the fungus on agar was most rapid at 26°–28° C. No growth took place at 36° C., and it was very slow at 7°–8° C. The colonies were hemispherical in shape and olivaceous in color. When the growth was submerged, they were darker. The hyphae soon became olivaceous to dark brown and, under suitable conditions, formed conidia. The conidiophores were produced singly on the vegetative hyphae as simple branches growing at right angles to the parent hyphae (Fig. 3, C). Each conidiophore was divided by septations into three or four cells. Anastomoses between hyphae and be-

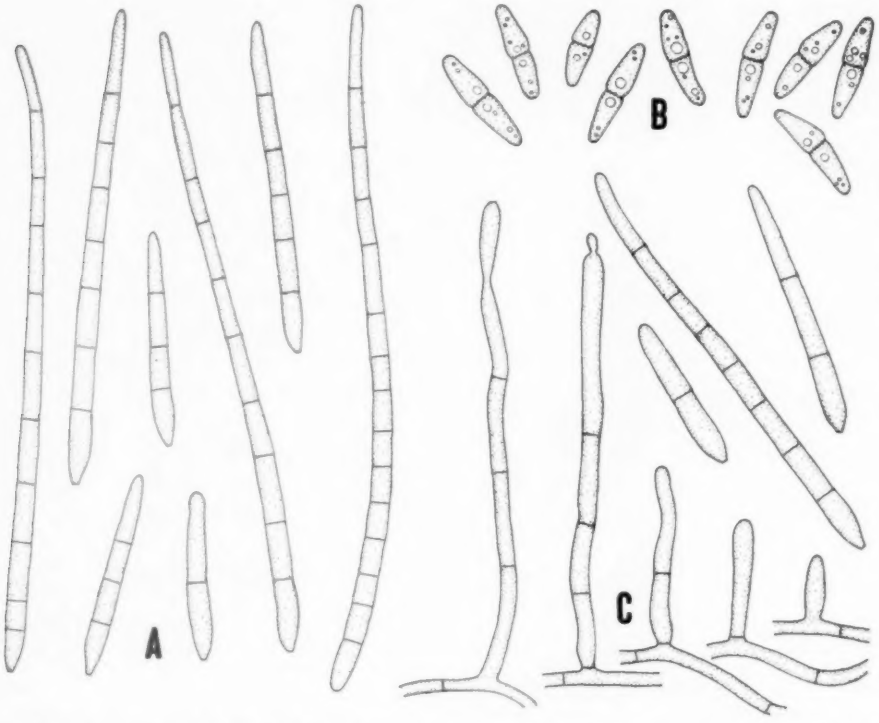


FIG. 3. A. Conidia of *Mycosphaerella puericola*. ×750. B. Ascospores of *M. puericola*. ×750. C. Different stages in the development of conidia of *M. puericola* in an ascospore culture. ×750.

tween hyphae and conidiophores were frequent. The conidia were borne singly at the apices of the conidiophores and were identical with those produced in the field on kudzu leaves. Conidial production was not abundant on any medium.

The fact that isolates from ascospores were identical in appearance and growth response in culture to those obtained from conidia and that the mycelium of the former produced identical conidia in culture is additional proof that these spores are different stages of the same fungus.

Primary infection of kudzu leaves in the spring is accomplished by

either ascospores or conidia from overwintered leaves infected during the previous season. Study of cleared pieces of kudzu leaves showed that stomata are relatively rare on the upper surface. Since infection takes place readily when inoculum is placed on the upper surface of the leaf, it seems probable that the fungus is capable of direct penetration of the epidermis. The mycelium is both intracellular and, at least in late stages, intercellular. Inoculation experiments showed that the incubation period is approximately two weeks. At the end of this time the occupied tissue becomes necrotic, and the fungus fruits in the dead tissue of the resulting leaf spot. The conidial fructifications consist of erumpent clusters of densely crowded, erect or suberect conidiophores arising from globose, pseudoparenchymatous stromata embedded in the mesophyll. The conidiophores usually are simple, 1–6-septate (commonly 3–4-septate), non-constricted at the septa, olive-brown, somewhat lighter in color and slightly attenuated at the apex, and measure 20–84 × 4–4.5 μ. Often one or more old conidial scars are visible. The conidial tufts are formed on both surfaces of the leaf but occur more commonly on the upper surface.

Conidia are formed abundantly on the host during wet weather and on leaves held in a moist chamber for 24 hours. The conidia (Fig. 3, A) are almost hyaline to pale olive, straight or more commonly curved, cylindric-obclavate, truncate or rounded at the base, rounded at the tip, distinctly tapering toward the apex and slightly near the base, 1–16-septate, non-constricted at the septa, and measure 27.3–126 × 3.2–5.3 μ (av. 72.7 × 4.3 μ). They are borne singly at the apices of the conidiophores. In the formation of a conidium the conidiophore produces a bud at the apex. This elongates and becomes septate to form the conidium. The developing conidium is marked off from the conidiophore by a constriction. At maturity it separates from the conidiophore at a cross wall laid down in this constriction. The truncate base of the conidium thus marks its point of attachment to the conidiophore.

The conidia produced on the host constitute a source of inoculum for repeated infections of new leaves throughout the growing season. The conidial stromata may overwinter on fallen leaves and produce conidia the following spring. The fungus, therefore, is capable of surviving from year to year in the conidial stage alone.

Spermogonia and perithecial initials develop within the lesions on moribund or dead leaves during the fall. Although they may be present on both surfaces of the leaf, they are much more abundant on the upper surface. In the earliest stages of their development they cannot be distinguished. Both structures originate from the internal mycelium as small stromata. They appear in the mesophyll just beneath the epidermis and usually are formed independently of the conidial stromata. The stromata enlarge until they raise the epidermis and finally become erumpent at the apex. The outer layers of cells are brown and thick-walled. Hyaline cells form a plectenchymatous or pseudoparenchymatous core. Although they

remain similar in external appearance, spermogonia and perithecia differ in further internal development. The hyaline cells in the interior of the developing spermogonium produce quantities of hyaline, bacilliform spermatia measuring approximately $3 \times 1\ \mu$. As the spermatia are formed the cells that produced them disintegrate, leaving the spermogonium at maturity composed of a thin wall, one or two cells thick, enclosing a mass of spermatia. At the protruding apex the spermogonium is provided with a pore through which the spermatia emerge. Spermogonia are irregularly globose and measure 42–67 μ (av. 52.7 μ) in diameter and 54–67 μ (av. 59.2 μ) in height.

Within the pseudoparenchyma filling the perithecial initial two or three enlarged ascogonial cells are formed. They are not noticeably more deeply staining than the surrounding cells but are conspicuous because of the single, extremely large nucleus in each cell. Trichogynes have been found projecting from the apex of the perithecial initial, but their connection with the ascogonia has not been traced. During the winter the perithecia continue to enlarge, and in the following spring a fascicle of asci pushes up from the base of the perithecium into the central pseudoparenchyma which is crushed and disintegrated. At maturity the perithecium is spherical with a short, ostiolate neck at the apex which protrudes through the epidermis. Perithecia measure 61–93 μ (av. 71.2 μ) in diameter and 70–115 μ (av. 86.7 μ) in height. The wall is composed of one to three layers of brown, thick-walled cells. The central cavity is occupied by a cluster of clavate, aparaphysate asci, each containing eight irregularly biseriate ascospores. The asci measure $44.8–60.8 \times 9.3–9.9\ \mu$. The ascospores (Fig. 3, B) are hyaline to pale green, 1-septate, slightly constricted at the septum, fusoid or clavate, straight or slightly curved, rounded at either end, and measure $14.3–24.2 \times 2.7–4.5\ \mu$ (av. $19.7 \times 4.2\ \mu$). Each cell of the ascospore usually contains a large globule near the septum and one or several smaller ones near the tip. When the perithecia are moistened, the ascospores are forcibly discharged. They germinate readily, sending out a germ tube from each end of the spore.

In 1947 mature ascospores were not found in field material until May. This was an unusually late season, however, and doubtless they usually develop earlier. When leaves were brought in from the field and kept in a moist chamber, ascospores developed in April.

The fungus probably is carried to new localities on planting stock. Although the crowns are dug and transplanted in late winter or early spring after the leaves have fallen, it is possible that fragments of old diseased leaves containing the sclerotia-like fruiting bodies of the fungus are transported with the young plants.

CONTROL

No studies on the control of angular leaf spot of kudzu have been made but a knowledge of methods of propagating this plant suggests certain precautions that might be helpful.

During the 1930 decade considerable seed was imported from Japan. Because seed has not been available from this source in recent years, propagation by means of crowns has become the accepted practice. It seems probable that seed may soon become more abundant and again come into common use. Kudzu seeds need scarification before planting and the method reported by Tabor (6), namely, soaking the seed in concentrated commerical sulfuric acid for one hour at 60° F., should serve not only to scarify the seed coats but to disinfect them as well. It is not known whether the fungus ever enters the seed, but this seems improbable. So far as known, it attacks only the leaf tissue.

The difficulty of completely removing the fungus from other propagating material is obvious. Johnson (4) recommends soaking the vine cuttings for 20 minutes to one hour or longer in a 0.1 per cent solution of potassium permanganate just before planting to stimulate root growth. It seems certain that this chemical at the recommended strength would have little if any fungicidal value. It is possible, of course, that other fungicides could be found that would be effective and not injure the tissue. Perhaps for the present it would be sufficient to use every precaution to free the propagating material of all old leaf tissue and soil particles that might harbor the fungus.

If, as now seems probable, this disease has been in the United States for a number of years without having attracted more attention, control measures, other than the precautionary ones suggested above, may not be needed. Yet in some instances observed the plants had lost a considerable proportion of their foliage. The fact that kudzu is a perennial and the planting remains in the same location for several years should offer excellent opportunity for the fungus to become more abundant and more destructive each year. On the other hand, the plant grows so rapidly that a severe epiphytotic would be required to destroy it.

DISCUSSION

Since this disease has been reported from Formosa (8) and China (7), it seems probable that it is widespread in the Orient. No specific reference to the occurrence of angular leaf spot in Japan has been found. All of the kudzu seed used in the United States from 1910 to 1940, or until the supply was cut off, is said to have come from Japan. It is not known to the writers whether some of this seed originated from Formosa, which has been classed by some Atlases as south Japan. At any rate the most logical explanation of the presence of this disease in the United States seems to be that it was imported with the seed. This suggests that the fungus is probably widespread in this country, especially in sections where the imported seed was planted. The recent report of its occurrence in Mississippi and the present report of its presence in Georgia and Alabama support such a supposition. It seems probable that this disease has been over-looked previously because of its similarity in appearance to certain stages of halo

spot. The kudzu plant grows so rapidly that the older leaves are more or less covered. The angular leaf spot develops rather slowly so that often it probably is not sufficiently abundant to attract attention until fairly late in the season.

SUMMARY

The symptomatology and etiology of a leaf spot of kudzu (*Pueraria thunbergiana* Benth.) herein designated as angular leaf spot, is described. The causal fungus appears to be identical with *Cercospora pueraricola* Yamamoto. Until recently the disease had been reported only from the Orient. A recent report by Bain and Presley, together with the present one, show that the disease is present in Mississippi, Georgia, and Alabama, and probably is much more widespread than was formerly supposed. Lesions, which are confined to the leaves, are 1 to 10 mm. in diameter, dark brown, often with a yellowish margin, and, during damp weather, with numerous conidial tufts largely confined to the upper surface. Lesions may coalesce and involve large areas of the leaf and cause defoliation.

The perfect stage of the causal fungus was found on overwintered kudzu leaves. The ascospore stage was isolated, and its appearance in culture and its pathogenicity prove that it is identical with the conidial stage associated with the disease on living leaves. The characteristics of the perithecial stage place the fungus in the genus *Mycosphaerella*, and it is described as new under the name *M. pueraricola*.

U. S. DEPARTMENT OF AGRICULTURE
AND
GEORGIA AGRICULTURAL EXPERIMENT STATION

LITERATURE CITED

1. BAIN, D. C. and J. T. PRESLEY. A leaf disease of kudzu new to the United States. U. S. Dept. Agr., Plant Dis. Reptr. **31**: 188–189. 1947.
2. HEDGES, FLORENCE. Bacterial halo spot of kudzu caused by *Bacterium puerariae* Hedges. Jour. Agr. Res. [U.S.] **36**: 419–428. 1928.
3. HIGGINS, B. B. Halo spot of beans and kudzu. Ga. Agr. Expt. Sta. Bul. 161. 1930.
4. JOHNSON, G. W. Methods listed for establishing kudzu by vine cuttings. Miss. Farm Res. 8. 1945.
5. LEONIAN, L. H. Physiological studies on the genus *Phytophthora*. West. Va. Agr. Expt. Sta. Scientific paper 11: 444–498. 1925.
6. TABOR, P. Observations on kudzu, Pueraria thunbergiana Benth., seedlings. Jour. Amer. Soc. Agron. **34**: 500–501. 1942.
7. TAI, F. L. Notes on Chinese fungi. VII. Bull. Chin. Bot. Soc. 2: 45–66. 1936.
8. YAMAMOTO, W. Cercospora from Formosa. I. Trans. Sapporo Nat. Hist. Soc. **13**: 139–143. 1934.

CANKER OF TUNG TREES CAUSED BY PHYSALOSPORA RHODINA

JOHN R. LARGE[1]

(Accepted for publication January 16, 1948)

In the late summer of 1944 a canker disease of tung nursery trees caused by *Diplodia theobromae* (Pat.) Nowell[2] (*D. natalensis* Evans) was found on about 300 trees in a tung nursery in Pearl River County, Mississippi. Later the same symptoms were observed in an extensive 2-year-old orchard in the same county. At four different points in this orchard one or two trees were severely affected and several had cankers on one or more branches. In October the disease was observed on several 8-year-old trees in another large Pearl River County orchard. Subsequently about 75 trees, affected in various degrees, were found in a newly planted orchard in St. Tammany Parish, Louisiana. During 1945 and 1946, infected trees have been found in four additional orchards in Pearl River County.

LITERATURE

Eddins and Voorhees (1) inoculated tung and 30 other host plants with mycelia from pure cultures obtained from pycnospores of the imperfect stages of *Physalospora zeicola*, *P. rhodina*, and *Diplodia tubericola*. Edson and Wood (2) reported *Diplodia natalensis* as a pathogen causing crown girdle of tung. Verrall (9), after inoculating small tung trees with 10 isolates, concluded that none of the isolates used was sufficiently pathogenic to cause more than small temporary cankers that soon callused over. Stevens and Wilcox (8) found that the pycnospores of many species of *Diplodia* from various tropical hosts in different parts of the world, were identical, and the cultures showed the unusual characteristics of growing at 37° C. and turning potato-dextrose agar pink at that temperature. Pole-Evans (4) called attention to the fact that *Diplodia natalensis* (*D. theobromae*) fruits more readily on apples than on citrus fruit. Ramsey *et al.* (5) inoculated sweet potato, apple, and orange with the onion *Diplodia*, and gave detailed description of the development of the disease on the apple.

SYMPTOMS

During late summer and early fall the disease as observed on tung in Mississippi and Louisiana was characterized by black sunken cankers on the trunks, limbs, twigs, shoots, or leaf petioles. Sometimes the canker girdled the trunk, killing the tree back almost to the soil level, after which

[1] Assistant Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Specific determination of the organism was made by John A. Stevenson, Division of Mycology and Disease Survey, U. S. Department of Agriculture.

new shoots developed just below the canker. In other cases only a single branch, such as the tip of the central leader, was affected. When a canker girdled the trunk or branch, the leaves wilted suddenly and the affected part died. The following spring many of the dead branches developed ash grey cankers with numerous black perithecia scattered over the surface. This is characteristic of *Physalospora rhodina,* as described by Voorhees (10).

In the fall dead branches were collected for study and pycnidia containing single-celled hyaline spores 13–27 $\mu \times 6$–15 μ, averaging 24 $\mu \times$ 14 μ and resembling spores of *Macrophoma,* were consistently observed on these branches. This material was studied a year later and several dark bicellular spores 19–24 $\mu \times 10$–12 μ, averaging 22 $\mu \times 11$ μ, were observed. Fawcett (3) stated that this change from single celled spores to bicellular spores is typical of the fungus *Diplodia natalensis* (*D. theobromae*).

Cultures from spores obtained from tung material, as well as tissue cultures from the margin of the canker and from the black pith of the diseased branch, developed dark mycelium which gradually turned the culture brown to dark brown, appearing black in mass, which is characteristic of cultures of the fungus *Diplodia natalensis* (*D. theobromae*). Cultures from these isolations were used for pathogenicity studies.

Perithecia which contained hyaline asci and ascospores characteristic of *Physalospora,* were observed on the ash grey twig cankers. The asci were 63–75 $\mu \times 20$–22 μ; the ascospores 15–24 $\mu \times 8$–9 μ. When sectioned transversely, the contents of the mature perithecia were almost snow white. Shear, Stevens, and Wilcox (6) consider this a characteristic which makes it possible to identify the genus *Physalospora* in the field.

PATHOGENICITY

Cankers of the disease have been produced on stems of one-year-old tung trees inoculated in the greenhouse with isolates of the fungus growing on agar medium. The pathogen was later reisolated from the inoculated stems. In all cases 10-day-old cultures of the fungus on potato-dextrose agar, in Petri plates, were employed for the inoculations. A block of the agar culture approximately a half inch square was applied to the surface of the tung stem, which had been injured by punctures with a sterile needle. The agar medium was then covered with wet cotton, tied around the stem with string and the entire stem tip inserted into a paper bag which was tied shut below the point of inoculation. The bags and cotton were removed three days after inoculation . Controls were treated similarly to the above, but sterile agar blocks were used. The results of the inoculations are shown in table 1.

The portion above the canker of the two trees first infected died within 3 days after inoculation and manifested the characteristic darkening of the

pith and ashy grey stem areas covered with black pycnidia which appeared snow white in cross section. The trees that became infected later developed symptoms of the disease very slowly. After three months the cankers formed on the inoculated stems were only 2 cm. long by 1 cm. wide. In no case did the canker girdle the stem. Evidently the difference in rate of development of the disease was due to the vigor of the inoculated plant. Voorhees (10) and Verrall (9) noted the slow extension of the infection on inoculated woody stems, due to the formation of callus tissue which prevented further spread of the fungus.

Reisolations of the fungus, three months after inoculation, from 10 cankers gave typical growth of *Diplodia* on potato-dextrose agar, identical with the parent cultures.

The injuries on the check trees healed within three weeks.

TABLE 1.—*Results of inoculating young tung trees with pure mycelial cultures of Diplodia theobromae*

Method of inoculation	Number of trees inoculated	Number of trees infected after 15 days	after 90 days	after 350 days
Mycelial cultures placed over wounds in the stem	60	2	28	36
Control: stem wounded and covered with sterile agar	10	0	0	0

EFFECT ON APPLE FRUITS

Ripe apples inoculated with the tung *Diplodia* developed typical black rot symptoms, which Ramsey *et al.* (5) have described as follows: "Apples inoculated through a side wound developed an extensive brownish-black lesion within a week, and ultimately the whole fruit was converted into a black, moderately firm mummy. Pycnidia were formed in the peel and the internal tissues were full of large hyphae, typical for *Diplodia.*" Single-celled hyaline spores were observed in the pycnidia, one week after inoculation, and dark colored bicellular spores were observed a month later. These results are in agreement with those of others (1, 5, 7, 9, 10) who have shown that strains of *Diplodia* from a wide variety of crop plants are similar morphologically and produce similar symptoms when cross-inoculated on a number of different host plants.

EFFECT OF TEMPERATURE ON CHROMOGENESIS

Stevens (7) found that cultures of *Diplodia* showed the unusual characteristic of growing at 37° C., and that many strains turned potato-dextrose agar pink at that temperature. Voorhees (10) observed that stock cultures of several races of *Physalospora rhodina* growing on potato-dextrose agar at approximately 17° C. showed chromogenesis. However, when subcul-

cultures from the stock cultures showing this character were made on potato-dextrose agar in Petri dishes and incubated at 35° C. or below, no color developed.

The cultures of *Diplodia* reisolated from inoculated tung trees showed chromogenesis when grown on cornmeal agar stored in a refrigerator at approximately 17° C., on Difco potato-dextrose agar in test tubes held at 37° C., and on potato-dextrose agar, made according to the formula of Stevens and Wilcox (8), held at 37° C. Cultures growing on rice medium at 37° C. and also at room temperature from 19 to 27° C., showed definite chromogenesis. The tung *Diplodia* grew more rapidly when incubated at 37° C. than at room temperature (table 2).

TABLE 2.—*Growth of tung Diplodia on potato-dextrose agar at different temperatures*

Temperature, degrees C.	Cultures	Average diameter of culture after		
		24 hours	48 hours	72 hours
	Number	*Mm.*	*Mm.*	*Mm.*
17	5	0	0	0
19-27	5	17	34	41
37	5	25	50	76

No spores were formed by the organism when grown on cornmeal agar, potato-dextrose agar, rice medium, sterilized tung stems, and sterilized tung leaves.

On the basis of the present studies, the writer considers the causal organism of the tung disease to be a strain of *Physalospora rhodina*, Cke., the perfect stage of *Diplodia theobromae* (Pat.) Nowell (*D. natalensis* Evans).

SUMMARY

Physalospora rhodina ex Cke., caused a canker disease of tung trees in several nurseries and orchards in Mississippi and Louisiana. The perithecia of this fungus were scattered singly in the bark of the host. When cut transversely, especially before the spores were fully mature, the contents of the perithecia had the white appearance characteristic of the genera *Botryosphaeria* and *Physalospora*. Pathogenicity was demonstrated by inoculation of tung seedlings, which developed both the *Physalospora* and *Diplodia* stages of the organism; cultures were reisolated from the *Diplodia theobromae* stage of the organism. In culture the tung *Diplodia* grew rapidly at 37° C. Cultures growing on rice medium at 37° C. and at room temperature showed definite chromogenesis. No spores were found on any of 5 culture media used. The tung *Diplodia* caused decay of ripe apples. Typi-

cal bicellular *Diplodia* spores were reisolated from the pycnidia, which developed in the peel of the inoculated mummified apple.

U. S. FIELD LABORATORY FOR TUNG INVESTIGATIONS,
BOGALUSA, LOUISIANA.

LITERATURE CITED

1. EDDINS, A. H. and R. K. VOORHEES. Physalospora zeicola on corn and its taxonomic and host relationships. Phytopath. **23**: 63-72. 1933.
2. EDSON, H. A. and JESSIE I. WOOD. Diseases of plants in the United States in 1936. U. S. Dept. Agric. Plant Disease Reporter, Suppl. **103**: 224. 1937.
3. FAWCETT, H. S. An offshoot and leaf stalk disease of date palms due to Diplodia. Phytopath. **20**: 339-344. 1930.
4. POLE-EVANS, I. B. On the structure and life history of *Diplodia natalensis*, n. sp. The cause of the "blackrot" of Natal citrus fruit. Transvaal Dept. Agr. Sci. Bul. **4**: 1-18. 1910.
5. RAMSEY, G. B., BARBARA C. HEIBERG, and J. S. WIANT. Diplodia rot of onions. Phytopath. **36**: 245-251. 1946.
6. SHEAR, C. L., N. E. STEVENS, and M. S. WILCOX. *Botryosphaeria* and *Physalospora* in the eastern United States. Mycologia **17**: 98-103. 1925.
7. STEVENS, N. E. Host relations in species of Diplodia and similar genera. Mycologia **33**: 69-73. 1941.
8. ———, and MARGARET S. WILCOX. The citrus stem end rot Diplodia, its life history and relation to *Sphaeropsis malorum*. Phytopath. **15**: 332-347. 1925.
9. VERRALL, A. F. A comparison of *Diplodia natalensis* from stained wood and other sources. Phytopath. **32**: 879-884. 1942.
10. VOORHEES. R. F. Life history and taxonomy of the fungus *Physalospora rhodina*. Fla. Agr. Exp. Sta. Bul. 371. 1942.

A LEAF-CURL DISEASE OF TOMATO

R. S. Vasudeva and J. Sam Raj

(Accepted for publication January 6, 1948)

A serious disease of tomato characterized by pronounced dwarfing of the leaves has been observed at Delhi and in other tomato growing areas for the last several years. The disease was particularly severe during the winter of 1946–47 and over 90 per cent of the plants in the early sown experimental plots of the Mycology Division of this Institute were found to be infected. In later sowings, however, the incidence of the disease was much lower. The plants, if infected while young, yield almost no crop. The disease occurs year after year and during 1943–44 and 1944–45 over sixty varieties of tomato which were under trial for their resistance to virus diseases were found to be more or less damaged by the disease. Considering its economic importance the study of the disease was undertaken.

SYMPTOMATOLOGY

Infected tomato plants of the variety Sutton's Early Market exhibit clearing of the veins, stunting and marked reduction in leaf size. At the same time the plant has typical mosaic symptoms which vary from mild to severe. The reduction in size of the leaves is more pronounced in the successive leaves and is accompanied by shortening of the internodes with the result that the leaves are crowded together. The leaflets are deformed and their margins may curl inwards or outwards. In a few cases the young leaflets show a tendency towards a corkscrew distortion with the tip forming a type of hook. The younger leaves are pale in color with light green and dark green areas. Sometimes the area between the veins may be completely yellow. Puckering of the leaflets is a common symptom of the disease and is very severe in some plants. The plant has a greater tendency to produce stunted lateral branches which impart a bushy appearance. The plants infected while young remain stunted and seldom attain a size of more than 10 to 15 inches. The disease induces partial or complete sterility of the infected plant, depending on the stage at which infection has taken place. In the cases of late infections the plant bears a few fruits but in earlier infections the symptoms are much more pronounced and the few flowers that are formed bear reduced floral structures which never attain normal size. They seldom open and soon drop off. The flowers that develop after infection are invariably sterile. The diseased plants usually develop purple patches especially on the older leaves, and these vary in intensity in different plants.

MATERIAL AND METHOD

Throughout the work, the diseased material was obtained from infected tomato plants of the variety Sutton's Early Market from the experimental

plots of the Mycological Division. The same variety of tomato was used throughout the investigation. The tomatoes, as well as the differential plants, were raised inside an insect-proof house and young, actively growing plants were used for the experiments. All the apparatus used was carefully sterilized as required for the individual experiment. For inoculation purposes, carborundum powder was used as an abrasive and grafting was carried out by the wedge method as well as by inarching.

TRANSMISSION OF THE DISEASE

All attempts to transmit the disease with diseased plant extracts to healthy tomato plants were unsuccessful. More than 50 attempts were made at different times but the inoculated plants failed to develop any symptoms of the disease. The disease was, however, successfully transmitted by grafting diseased scions to healthy stocks of tomato plants. Both the wedge-graft method and inarching successfully transmitted the disease within three weeks (Fig. 1, A). New shoots developed about seven to ten days after the graft. The healthy stocks first exhibited symptoms of mosaic which were accompanied by a stunting of the plant identical with that of naturally infected plants.

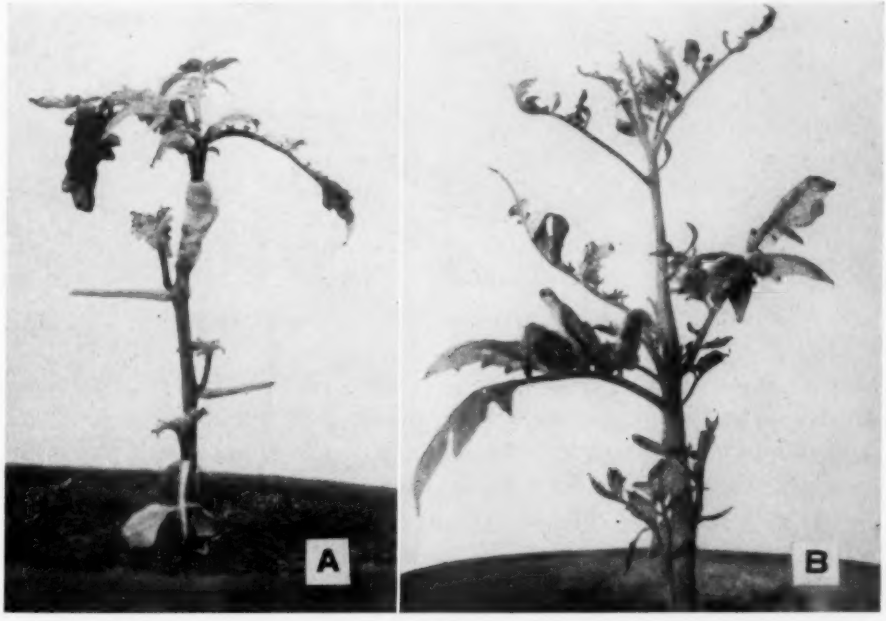


FIG. 1. Tomato plants in which the leaf-curl disease has been transmitted by grafting (A) and by white flies (B).

Transmission by insects. In order to determine the vectors responsible for transmission of the disease a careful survey of the insects present in the field at the time of the appearance of the disease was undertaken and it was found that two types of insects—namely, the capsid, *Engytatus tenuis* Reut.,

and the white fly, *Bemisia gossypiperda* M. Th.—were predominant. It was also observed that during October and November the disease was spreading rapidly in the fields and that the incidence of the two types of insects was very high. It was, therefore, considered necessary to determine whether either of these insects was responsible for the transmission and rapid spread of the disease. The insects used for transmission work were first fed on diseased plants for 24 hours with the help of microcages similar to those described by Storey (1928) in connection with his transmission studies of maize streak virus. After the insects fed on diseased plants they were released on healthy tomato plants inside glass lamp chimneys. The top of a chimney had been covered by muslin. The insects were allowed to feed on healthy tomato plants for two and three days, after which the surviving insects were killed by spraying. The transmission experiments were repeated several times with five to eight insects on each plant. The plants on which capsids had been released never developed any symptoms of the disease. In tests conducted at different times 30 to 100 per cent of the plants on which white flies had been released developed typical symptoms of the disease within 15 to 25 days, depending on the temperature prevalent during the experimental period. During the winter months 25 days were required for the manifestation of symptoms, whereas in March the symptoms appeared in 15 days. Figure 1, B shows a tomato plant infected by viruliferous white flies.

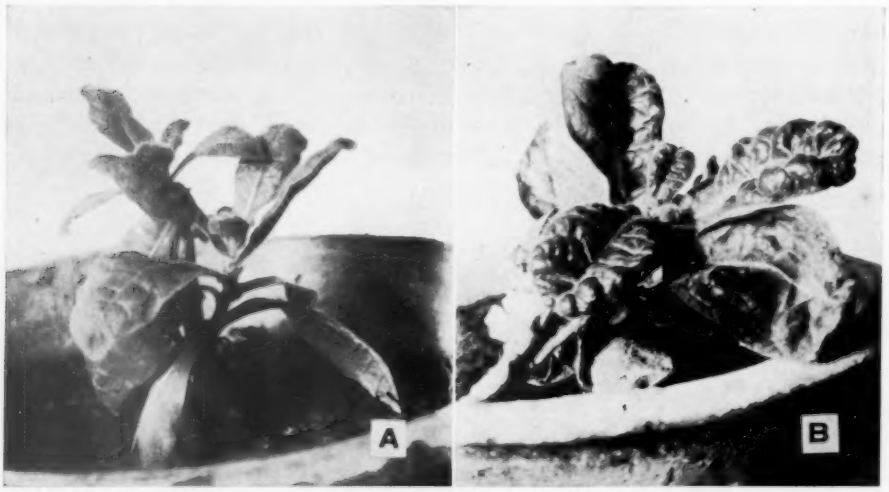


FIG. 2. Infected White Burley tobacco plants showing mild type of symptoms (A) and pronounced leaf-curl symptoms (B).

REACTIONS OF DIFFERENTIAL HOSTS

The disease was successfully transmitted by grafting to *Nicotiana tabacum* L. varieties White Burley, German Samsun, and Harrison's Special, *Solanum tuberosum* L. variety Craig's Defiance, *Datura stramonium* L.,

Nicotiana sylvestris Spegaz. and Comes, and *Nicotiana glutinosa* L. The reactions described are those observed during January to April at Delhi in the insect-proof house in the presence of adequate light and humidity.

A considerable variation was observed in the symptoms produced on *Nicotiana tabacum* var. White Burley. All the plants invariably showed a type of yellow mottling, but the subsequent symptoms differed in the intensity of stunting as well as in the reduction in size and curling of the leaves in different plants. The leaf tips in all cases point downwards. Symptoms may vary considerably in type. In one type the stunting of the plant is not pronounced but the leaf margins tend to curl downwards. In all other types stunting of the plant as well as shortening of the internodes and reduction of the leaf size are present in varying degrees. Vein-banding, rolling downwards of the leaf margins, and thickening of the veins also are common features. In some plants the leaf surface appears wavy (Fig. 2, A), but in many plants puckering is present. This may be mild and confined only to

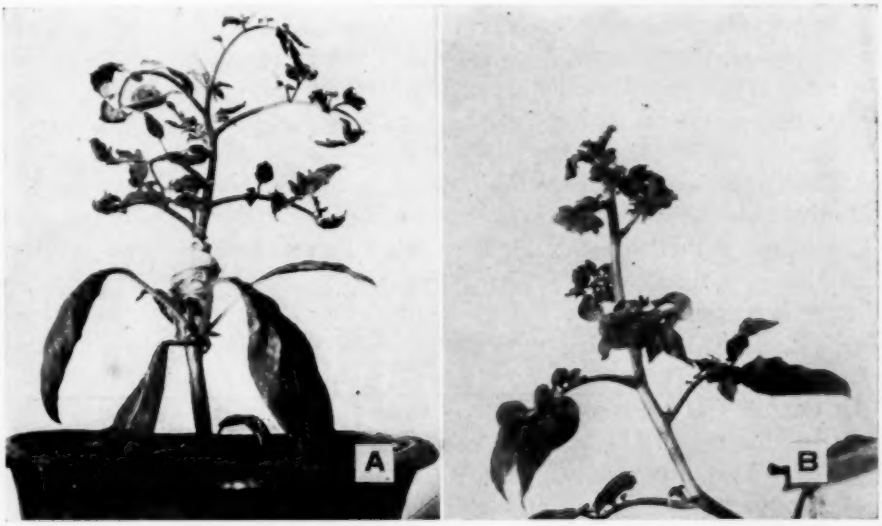


FIG. 3. A. *Datura stramonium* grafted with diseased tomato shoot. B. Shoot of an infected potato plant.

portions of the leaf, or may be general and highly pronounced. Clearing of the veins, when present, is only transient. In some plants the leaves are thick and brittle and enations in the form of small dark-green thickenings of the veins are present (Fig. 2, B).

The experimental data presented indicate that there may be a number of strains of tobacco leaf-curl virus responsible for the development of the leaf-curl disease in tomato. If this is true, it appears that the mild as well as the severe forms produce almost similar symptoms in tomato. A large number of grafting experiments indicate that the virulence of certain strains of the virus diminishes during passage through tomato, and when brought

back to tobacco the strains produce mild reactions. For instance, the strain that causes the enation type of leaf curl, when transmitted to a White Burley tobacco plant through tomato, produced symptoms resembling the mild type. The enations which are a characteristic feature of the type were absent.

In the German Samsun variety of tobacco the symptoms such as stunting of the plant, shortening of the internodes, reduction in the size of the leaf, as well as yellow mottling, are very pronounced, but the curling of the leaf margins and puckering are absent. In the variety Harrison's Special the symptoms are more or less similar to the above but the mottling is not pronounced and there is a tendency for the leaf margins to curl downwards. *Nicotiana sylvestris* reacts only mildly to the disease, yellow mottling and vein-banding being the prominent symptoms. In *Nicotiana glutinosa* L. there is a general yellow mottling, dwarfing, and cupping of the leaves.

Datura stramonium L. reacts violently to the disease: stunting is accompanied by a general yellow mottling, dwarfing, curling and corkscrew distortion of the leaves (Fig. 3, A).

In *Solanum tuberosum* L. (var. Craig's Defiance) stunting of the plant is accompanied by mottling, dwarfing, and thickening of the leaves, which are more or less crowded together and appear crumpled (Fig. 3, B).

DISCUSSION

The symptoms on differential hosts, particularly those on tobacco, indicate that this disease of tomato is caused by the tobacco leaf-curl virus since the curling of the leaves and development of enations are very typical of this disease on tobacco. This view is further confirmed by the fact that the disease is transmitted by *Bemisia gossypiperda*, vector of the tobacco leaf-curl virus. This virus has been experimentally transmitted by a number of workers, *e.g.*, Thung (9), Storey (8), Pal and Tandon (4), and McClean (3), but natural occurrence of the disease on tomato has been mentioned only by Pruthi and Samuel (5). The symptoms of the disease as it occurs on tomato have not, however, been fully described by the authors. Bertus (2) records the occurrence of a leaf curl and stunting disease of tomato which is transmitted by white flies and grafting but not through sap. It is likely that the disease that Bertus described is caused by the tobacco leaf-curl virus. The natural occurrence of the disease in wild varieties of tomato has been observed in Nyasaland (1).

As early as 1932 Thung (9) described three types of leaf curl on tobacco. The presence of more than one strain was also observed by Storey (7), when he found that there was considerable variation in the severity of the symptoms produced. In his transmission experiments his tobacco plants generally developed the disease only mildly. He suspected that the variations in the individual reactions of the plant may play a part and also that there may be a number of strains of the virus of varying virulence. Pal and Tandon (4) in their studies on tobacco leaf curl have differentiated five different types of leaf-curl virus, but from our observations it appears that a

much wider variation exists. McClean (3) states that in the course of the transmission of leaf curl of tobacco, a new type of the disease arose that produced milder symptoms on tobacco and other hosts. He distinguished severe, mild, and latent forms and stated that the latter two had their origin in plants infected with severe leaf curl. He also established the fact that in the process of transmission of virus from a plant with severe leaf curl plants may acquire weaker forms of virus which produce a milder form of disease.

The suggestion of Storey that the variations in the individual reaction of the plant may play a part in the variation of the severity of the disease also must be considered. Moreover, there is a wide variation in the symptoms produced in different varieties. In our experiments it was observed that the tobacco plants of variety White Burley when grafted to other varieties of tobacco plants already infected with leaf-curl virus developed different symptoms.

SUMMARY

A virus disease of the tomato which causes dwarfing, puckering of the leaves, vein-clearing, excessive branching, and stunting of the plant has been studied.

The disease can be transmitted by grafting and by *Bemisia gossypiperda* but not by inoculation with juice of infected plants.

The causal virus has been identified as that of tobacco leaf curl and inoculations to tobacco indicate that a number of strains of this virus may be involved but that all produce almost identical symptoms on tomato.

INDIAN AGRICULTURAL RESEARCH INSTITUTE,
NEW DELHI, INDIA.

LITERATURE CITED

1. ANONYMOUS. Virus diseases of tobacco in Nyasaland. Nyasaland Dept. Agr. Bul. 2. 1932.
2. BERTUS, L. S. Plant Pathology. Adm. Rept. Dir. Agr. Ceylon for 1942. p. D 5. 1943.
3. MCCLEAN, A. P. D. Some leaf-curl diseases in South Africa. Dept. Agr. and For. S. Africa Sci. Bul. 225. 1940.
4. PAL, B. P., and R. K. TANDON. Types of tobacco leaf-curl in Northern India. Indian Jour. Agr. Sci. 7: 363–393. 1937.
5. PRUTHI, H. S., and C. K. SAMUEL. Entomological investigations on the leaf-curl disease of tobacco in Northern India. IV. Transmission of the disease by white-fly (*Bemisia gossypiperda*) from some new alternate hosts. Indian Jour. Agr. Sci. 11: 387–409. 1941.
6. STOREY, H. H. Transmission studies of maize streak disease. Ann. Appl. Biol. 15: 1–25. 1928.
7. ———. Leaf-curl of tobacco in Southern Rhodesia. Rhodesian Agr. Jour. 29: 186–192. 1932.
8. ———. Virus diseases of East African Plants. II. Leaf-curl disease of tobacco. E. African Agr. Jour. 1: 148–153. 1935.
9. THUNG, T. H. De Krul-en Kroepoek-zeikten van tabak en de oorzaken van hare verbreiding. Meded. Proefst. Vorstenlandsche Tabak 72: 1–54. 1932.

INOCULATION EXPERIMENTS WITH POLYPORUS SCHWEINITZII[1]

SIGMUND BERK[2, 3]

(Accepted for publication January 10, 1948)

Polyporus schweinitzii Fries is considered primarily as a wood destroying organism that causes a root and butt rot in conifers. Although the literature gives a wide range of coniferous hosts for the fungus (7), there are only two reports of its occurrence on hardwoods. Rhoads (6) found it on a charred stump of *Eucalyptus globulus* and Hubert (4) reported it on red gum and oak.

This fungus has been found by Dr. H. H. York to be very destructive on forest plantings of white and red pine on the watershed of Hemlock and Canadice Lakes, N. Y. The land, originally forested with mixed hardwoods and conifers (chiefly white pine and hemlock), was cleared about 75 years ago for cultivation of beans, potatoes, wheat, oats, and timothy. The coniferous plantations were established about 10 to 25 years ago on old abandoned fields. *Polyporus schweinitzii* has been active in some of these forest plantings for at least 15 years. The fact that this fungus is so widespread suggests the following questions: Have the infections occurred from outside sources of spores of the fungus, or has this organism persisted saprophytically in the soil or as a weak parasite on the roots of hardwoods and field plants? The writer undertook a series of experiments to determine whether or not the fungus can parasitize the roots of various seedling hardwoods and herbaceous plants.

INOCULATLON OF SEEDLINGS GROWN IN POTS

Series A, Hardwood Seedlings. Soil used in Series A was obtained from the upper 8 inches of a forest planting of white pine near Springwater, N. Y., where *Polyporus schweinitzii* is widely epidemic. This soil had a pH of 6.0 and a colloidal content of 21 to 50 per cent. The soil was disinfected by wetting with a solution of formalin (1 oz. to a quart of water). The disinfectant was permitted to vaporize from the soil by occassional raking and exposure to air and sun for 3 days.

The inoculum used in Series A to D inclusive was prepared by filling 10-cm. Petri plates two-thirds full of finely divided pine needle duff from forest plantings of white pine near Springwater, N. Y. This duff, saturated with a 2 per cent solution of Fleischmann's Diamalt, was autoclaved for

[1] A portion of a dissertation presented to the faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Master of Arts.

[2] Present address, Pitman-Dunn Laboratory, Frankford Arsenal, Philadelphia, Pa.

[3] The writer wishes to express his appreciation to Dr. H. H. York under whose direction this investigation was carried out and to Dr. W. N. Ezekiel for valuable criticism of the manuscript.

30 minutes at 15 lb. steam pressure and inoculated at the center of the dish. After 2-4 weeks incubation at room temperature in diffused light, the fungus grew well through the duff, and the inoculum was ready for use. Unless stated otherwise, variant No. 12 of *Polyporus schweinitzii* was used in this study.

Four 6-inch clay pots comprised a series, two pots with treated soil and two with untreated soil. One pot with untreated and one with treated soil were inoculated just prior to planting by the addition of needle-duff inoculum from one Petri plate. Equivalent amounts of the noninoculated needle-duff were added to one pot with untreated and one with treated soil. Pots of soil that were inoculated with the fungus prior to planting received a second similar inoculation in the soil, 25 weeks after the hardwood seeds were planted.

The following seeds, which were not surface-sterilized, were sown on the surface of each pot and covered with soil: American elm (*Ulmus americana L.*), Scotch elm (*Ulmus glabra Huds.*), and silver maple (*Acer saccharinum L.*). All seeds and seedlings in Series A to F inclusive were grown in the greenhouse.

A striking difference in growth was observed in the hardwood seedlings after 11 weeks (Fig. 1). Dwarfing occurred in the plants grown in formal-

FIG. 1. Seedlings of American elm in (A) nontreated, noninoculated forest soil, (B) formaldehyde treated soil inoculated with *Polyporus schweinitzii*, (C) nontreated soil inoculated with *P. schweinitzii*, and (D) formaldehyde treated soil without fungus inoculum.

dehyde treated soil — in length of shoot and root systems, and in number and size of leaves per plant.

Isolations were made from the roots after 31 weeks when there were no open lesions of decay but portions of lateral roots had been killed. The infected roots were surface-sterilized by the following methods: a 2-minute

immersion in an aqueous solution of 0.1 per cent mercuric chloride, followed by at least 3 rinses in sterile distilled water; 5 to 20 seconds immersion in Superoxol (30 per cent hydrogren peroxide); and a 2-minute dip in 3 per cent hydrogen peroxide. The surface-sterilized roots were plated in tubes of 2 per cent Fleischmann's malt agar medium. A 2-minute application of 0.1 per cent aqueous solution of mercuric chloride with 3 rinses in sterile distilled water was found to be the most reliable root surface sterilization method. Discolored, swollen, dwarfed, and killed roots did not yield a single positive culture of *Polyporus schweinitzii*, but did yield species of *Fusarium, Trichoderma, Alternaria, Macrosporium,* and other fungi.

Series B. Lupine and American Elm Seedlings. Six-inch clay pots were filled with 1 part soil from Springwater, N. Y., 5 parts leaf humus, and 1 part sand. Inoculum from a 10-cm. Petri dish two-thirds full of pine needle duff saturated with 2 per cent Fleischmann's malt was added to each pot. The pH of this soil mixture after autoclaving was 6.4.

American elm and lupine (*Lupinus albus* L.) seeds were surface-sterilized by immersion for 12 to 45 minutes in an aqueous solution of calcium hypochlorite, somewhat similar to the method used by Wilson (8). The excess disinfectant was not washed from the seeds, since it does not interfere with germination. The pine needle duff in the pots was inoculated with *Polyporus schweinitzii* when the surface-sterilized seeds were sown.

Root isolations from lupine were made 55 days after sowing and from American elm 75 to 157 days after sowing. The lupine seedlings had slight lesions on the root crown and the tip of the tap root was killed. A few of the elm seedlings had lesions on the primary roots. *Polyporus schweinitzii* was not isolated from the diseased roots.

Series C, American Elm inoculated with Variants of Polyporus schweinitzii. Four-inch clay pots were filled with potting soil and a mixture of 2 parts potting soil to 1 part finely ground pine needle duff. The inoculum, prepared in Petri dishes as described previously and consisting of variants No. 10, 12, and 40E of *Polyporus schweinitzii* alone and in various combinations, was added to each pot at the time of sowing the surface-sterilized American elm seeds. Variants No. 10 and 12 were obtained by Childs (2) from infected *Pinus strobus* L., Springwater, N. Y., and No. 40E from *Pinus mughus,* Central Experimental Farm, Ottawa, Ontario. Variants of *P. schweinitzii* were used in this series, since it was thought that variant No. 12 used in series A and B might have been weakened by long and continued growth on artificial media or that possibly it was not a virulent "race" of the parasite. Isolations made from diseased roots after 12 weeks growth were negative for *P. schweinitzii.*

Series D, Liriodendron tulipifera Seedlings. Three-inch seedlings of *Liriodendron tulipifera* were transplanted into six-inch pots containing potting soil. The roots of each plant were surrounded by pine needle inoculum of variants No. 12 and 40E alone and in combination. Isolations

made from rotted primary roots after 10 weeks growth were negative for *Polyporus schweinitzii* and yielded primarily species of *Fusarium*.

Growth Rate of Fungi Isolated from Roots. The growth rates on 2 per cent Fleischmann's malt agar of a species of *Fusarium* and one of *Penicillium* isolated from infected roots were compared with that of variant No. 12 of *Polyporus schweinitzii*. The species of *Fusarium* grew twice as fast and the species of *Penicillium* three times as rapidly as *P. schweinitzii* on the malt agar medium. These results indicate the difficulty of isolating *P. schweinitzii* when rapidly growing soil organisms are present.

INOCULATION OF SEEDLINGS GROWN UNDER ASEPTIC CONDITIONS

Series E, Lupine and Bush Beans Grown in Pine Needle Duff. Test tubes (38 × 200 mm.) were one-quarter filled with finely ground pine needle duff saturated with 2 per cent Fleischmann's malt and autoclaved 30 min. at 15 lb. steam pressure. Surface-sterilized seeds of lupine (*Lupinus albus* L.) and bush bean (*Phaseolus vulgaris* var. *humilis* Alef.) were transferred to the pine needle duff in the test tubes. The duff was inoculated with variant No. 12 of the fungus, before, after, and at the same time as sowing the seeds.

The lupine control plants survived 40 days while the bush beans lasted 47 days under the growing conditions of this series. Autoclaved pine needle duff was somewhat toxic to the seedlings as evidenced by the reaction of the roots to the medium; *i.e.*, the appearance of adventitious roots above the surface of the duff. Examination of lupine roots 21 to 40 days after sowing and bush beans 28 to 43 days after sowing showed that the root crown and lower part of the stem had lesions, while the primary and secondary roots were necrotic. Severest infection occurred where the fungus mat was in direct contact with the root system. Although shoot development was good, the root system was no longer than 3 or 4 cm. and consisted of a thick primary root with few laterals. Diseased roots were grayish-black or dark brown and almost completely rotted by the fungus (Fig. 2). Isolations were made by dipping diseased portions of roots in 95 per cent ethyl alcohol and plating on malt agar. *Polyporus schweinitzii* was isolated from the roots of lupine and bush beans.

Root material was preserved in dilute formalin and fixed in a saturated aqueous solution of salicylic acid plus chromic sulfate and formaldehyde (3). A histological study of roots from this series revealed the presence of fungus hyphae in the cortical cells of lupine (Fig. 2). However, it is not known whether the fungus penetrated the roots while they were alive or after their death.

Series F, Lupine Seedlings Grown in Soil. Surface-sterilized lupine seeds were grown under aseptic conditions in test tubes (38 × 200 mm.) containing a mixture of 6 parts of potting soil and 1 part of pine needle duff saturated with distilled water and autoclaved one hour at 15 lb. steam

pressure. The cultures were inoculated with variant No. 12 of the fungus at the time of sowing the seeds.

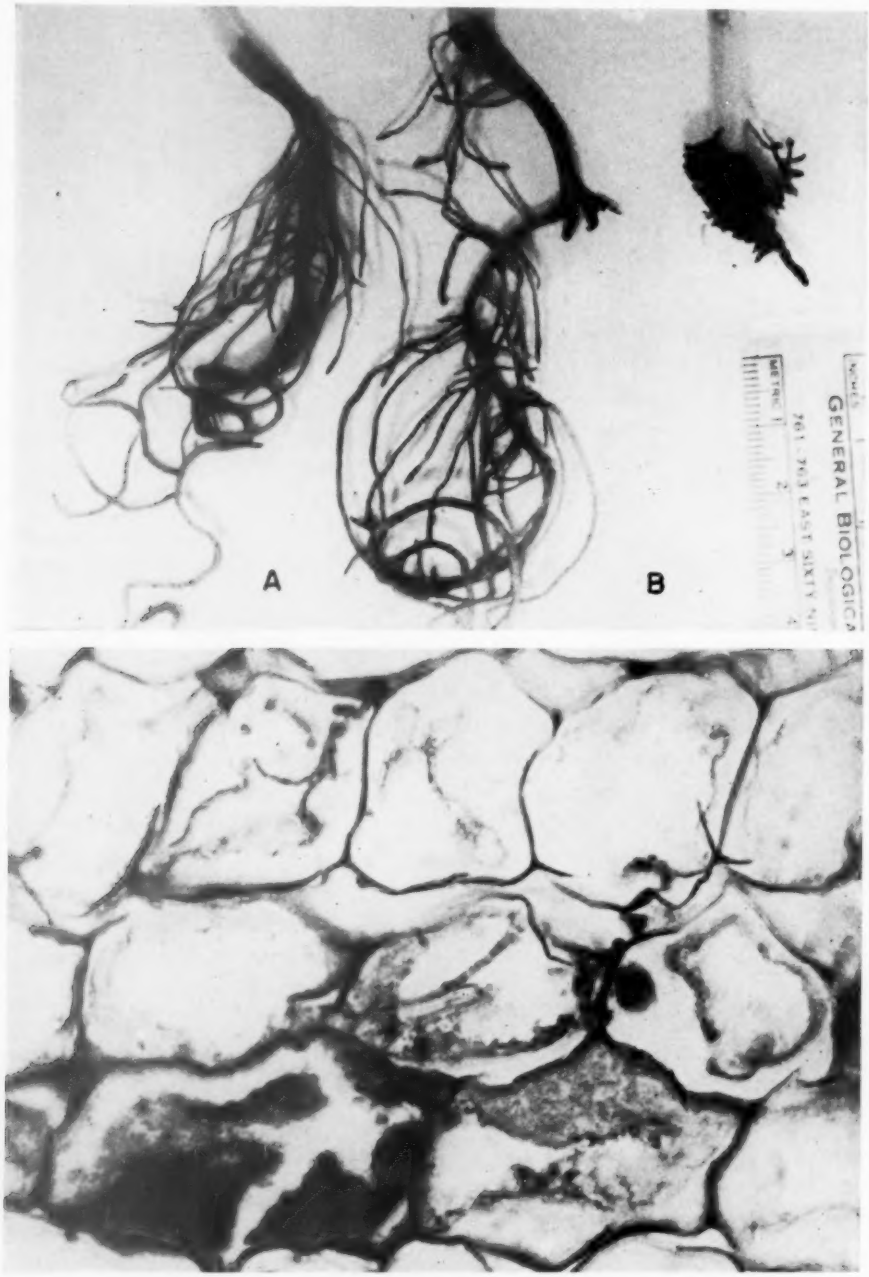


FIG. 2. Necrotic roots of lupine caused by *Polyporus schweinitzii*. Above: A from Series F grown in autoclaved soil; B from Series E grown in pine needle duff saturated with 2 per cent Fleischmann's malt. Below: Photomicrograph showing hyphae of *P. schweinitzii* in cortical cells of *Lupinus albus* L. (Mag. 1220×).

After 3 weeks' growth, discolored areas were present on the root crown and primary root. Laterals near the surface of the soil and in contact with the pine needle inoculum were killed (Fig. 2). *Polyporus schweinitzii* was isolated from such dead laterals. The fungus was not isolated from the primary root or from secondary roots more than 2 cm. below the surface of the soil.

GROWTH OF P. SCHWEINITZII IN STERILIZED SOILS IN THE LABORATORY

Boyce (1) questioned the ability of the fungus to grow in natural soil. To study the growth or survival of the fungus in soils, horizontal glass tubes (25 × 320 mm.) were utilized. The following types of soil mixtures saturated with distilled water were added to the tubes: Soil from Springwater, N. Y. (Series A); 4 parts soil from Springwater, N. Y., plus 1 part finely ground pine needle duff; and potting soil from Series C and D. After autoclaving for one hr. at 15 lb. steam pressure, the soils in the tubes were inoculated at one end and incubated in the laboratory at 22° C. in a horizontal position in total darkness.

Table 1 shows the rate of growth of *Polyporus schweinitzii* in the three

TABLE 1.—*Rate of growth of Polyporus schweinitzii in soils in glass tubes*

Type of soil	pH	Days after inoculation			
		26	36	43	53
		Growth in mm.			
From Springwater, N. Y.	5.8	120	170	190	320 [a]
4 parts soil from Springwater, N. Y., plus 1 part pine needle duff	5.1	170	240	.....	320 [a]
Potting soil	6.1	10	20	20	22

[a] Mycelium completely filled the 320-mm. tubes.

types of soil mixtures. It grew most rapidly in soil from the Springwater region plus pine needle duff. *P. schweinitzii* was isolated when the mycelium had grown through the soil to the opposite end of the tube.

Attempts to isolate the fungus from inoculated soils (Series A to D) in pots in the greenhouse failed.

DISCUSSION

The results of this investigation indicate that *Polyporus schweinitzii* is not capable of parasitizing roots of the hardwood seedlings under the conditions of this study. Although there were signs of root decay and of dead laterals, isolations from such regions did not yield the fungus.

In autoclaved soils of the various series inoculated with the fungus, the organism is not only capable of surviving but of actually making rapid growth in some. However, in sterilized soils, the organism does not en-

counter normal soil competing fungi which enter when the soil is exposed to the atmosphere. Species of *Fusarium*, *Penicillium*, and other fungi isolated from the roots grew more rapidly than *Polyporus schweinitzii* in culture. It is possible that the inoculum may have been completely overrun by such fast growing forms and the fungus suppressed before it could penetrate host tissue. Assuming the destruction of primary inoculum and the possibility that *P. schweinitzii* can parasitize only when the host has reached a certain stage of development, the soil in Series A was inoculated a second time. Attempts to isolate the fungus from the roots of the hardwood seedlings growing in the reinoculated soil also failed.

Rayner (5) states that Melin working with mycorrhizal fungi found *Fusarium* frequently outgrowing the true endophyte. It is possible that *Polyporus schweinitzii* penetrating host tissue offered an atrium for a secondary fungus like *Fusarium* which crowded out the primary parasite because of a faster growth rate. Considering the large number of isolations of *Fusarium* obtained, this concept may be worth further testing.

In autoclaved pine needle duff saturated with 2 per cent malt (Series E), conditions of growth apparently were optimum for the fungus but very unfavorable for the host. It seems from the work of Wean (7) that the fungus produces succinic acid which may weaken or kill root tissue and allow the hyphae to penetrate the cells with little difficulty. Wean (7) found that the fungus entered directly through living epidermal and cortical cells and through the base of lateral roots of *Pinus strobus* L. The author also found fungus hyphae in the cortical cells of lupine in Series E.

Results from inoculations in Series F seem to indicate that the fungus was weakly parasitic. The writer favors Hubert's (4) views that the organism may become parasitic only when the host is growing under very unfavorable environmental conditions. Infection of lupine roots was obtained under aseptic conditions in the absence of competing soil fungi. However, it was impossible to isolate the fungus from the roots of lupine plants growing in soil exposed to the atmosphere. It is possible that *Polyporus schweinitzii* may have been overgrown or suppressed by more rapidly growing organisms which are present in soils exposed to the air.

The results of these experiments do not preclude the possibility that the organism may live in agricultural soils for a long time after the land has been cleared, especially when the soil is rich in humus materials.

SUMMARY

Herbaceous and hardwood seedlings grown in various soils were inoculated with *Polyporus schweinitzii* in order to test the ability of the fungus to parasitize living root tissue. The *Polyporus* could not be reisolated from roots, possibly because a species of *Fusarium* and one of *Penicillium* obtained from infected roots have growth rates that are much greater, on 2 per cent Fleischmann's malt agar, than the growth rate of *P. schweinitzii*.

Infection characterized by necrotic lesions was obtained in bush bean and lupine seedlings grown under aseptic conditions in a medium of autoclaved finely ground pine needle duff saturated with 2 per cent malt and inoculated with the fungus. Lupine grown in soil under aseptic conditions showed a slight infection of lateral roots near the surface of the soil.

The fungus can survive and grow rapidly in certain types of sterilized soil. Best growth was obtained in soil from Springwater, N. Y. (pH of 5.8 and a colloidal content of 21–50 per cent). The most rapid growth was in soil from the Springwater region containing a small amount of pine needle duff.

BOTANICAL LABORATORY
UNIVERSITY OF PENNSYLVANIA,
PHILADELPHIA, PENNSYLVANIA.

LITERATURE CITED

1. BOYCE, J. S. Forest pathology. 600 pp. McGraw-Hill Book Co., Inc., New York and London. 1938.
2. CHILDS, T. W. Variability of *Polyporus schweinitzii* in culture. Phytopath. 27: 29–50. 1937.
3. COHEN, I., and K. D. DOAK. The fixing and staining of *Liriodendron tulipifera* root tips and their mycorrihizal fungus. Stain Technol. 10: 25–32. 1935.
4. HUBERT, E. E. An outline of forest pathology. 543 pp. J. Wiley & Sons, Inc., New York; Chapman & Hall, Ltd., London. 1931.
5. RAYNER, M. C. Mycorrhiza. 246 pp. Weldon & Wesley, Ltd., London. New Phytologist Reprint No. 15. 1927.
6. RHOADS, A. S. Some new or little known hosts for wood-destroying fungi. III. Phytopath. 11: 319–326. 1921.
7. WEAN, R. E. The parasitism of *Polyporus schweinitzii* on seedling *Pinus strobus*. Phytopath. 27: 1124–1142. 1937.
8. WILSON, J. K. Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. 2: 420. 1915.

WHITE ROT OF SHALLOT

E. C. Tims

(Accepted for publication January 12, 1948)

INTRODUCTION

White rot (*Sclerotium cepivorum* Berk.) of shallot, garlic, and onion was first observed in Louisiana in 1942 (21). The only known infestations at present are on a few small farms scattered in that area of the State which extends for about ten miles along the east bank of the Mississippi River about halfway between Baton Rouge and New Orleans. Shallots are grown intensively in this area along with some garlic and onions. Most of the farms are small and rotation is almost impossible. Although the infected areas are rather limited now, white rot is a potential threat to the entire shallot, garlic, and onion growing business in the southern part of the State. These crops are grown in the cooler season of the year when conditions are most favorable for the development of the disease.

This paper includes studies of white rot as it occurs on shallot in Louisiana. The pH relationships of the causal fungus were studied in culture on liquid and solid media. Control measures tested in naturally infected soil under field conditions included (1) the application of lime and other chemicals to the soil and (2) use of certain seed treatment fungicides. A number of shallot varieties were tested for resistance to white rot.

SYMPTOMS ON SHALLOT

Affected plants first show a slight wilting of one or more leaves. The adjoining leaves gradually wilt and the tips turn yellow and die back. The plants may gradually decline for several days or there may be a rapid wilt and collapse. Shallot bunches often remain severely diseased on one side for some time while the other side may be healthy.

The stunted, yellow, wilted, diseased and dead plants are conspicuous among the dark green healthy bunches. Infected plants can be pulled from the soil easily because of the decayed root systems. The roots rot off at the crown, and the basal portions of the scales become softened and slough off. The fungus often causes a semi-watery decay of the scales not unlike that caused by *Sclerotinia sclerotiorum* on other vegetables. The white to grayish mycelium of the fungus develops on the diseased portions of the plant below the soil line, and soon large numbers of small, black sclerotia appear. Typical infected shallots are shown in figure 1. Most of the affected shallot plants die before any bulbs are formed. Occasionally some bulbs are formed if infection occurs late in the season. However, such bulbs, even if slightly infected, almost invariably decay within a few weeks after harvest.

SEASONAL DEVELOPMENT

Walker (30) demonstrated that in general the disease on onion was limited to temperatures below 24°C, and developed best between 10° and 20° C. A similar relationship appears to be true in shallots. The earliest appearance of the disease observed in Louisiana was the first of December, and the period most favorable for infection lasts until about March first. White rot in Louisiana is definitely confined to the cooler seasons.

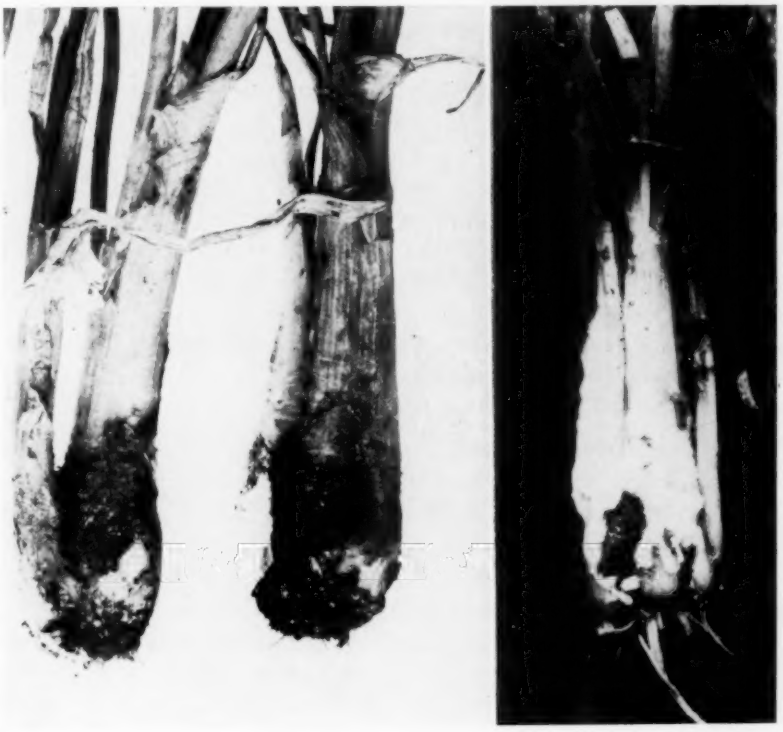


FIG. 1. Shallot plants infected with white rot. Bunch on right shows white mycelium on surface.

Under certain conditions shallot plants may become infected, collapse, and die within 20 days. Healthy transplants (green shallot plants) were set out in naturally infested soil in December, 1942. Twenty days later several of the plants were dead. Sclerotia had already formed on the basal portions of the dead plants.

DISTRIBUTION AND IMPORTANCE

White rot of onion and some other closely related plants has been known in Europe since the disease was first described by Berkeley (4) in 1841. According to Walker (29), the disease is commonly destructive to onion and leek in the British Isles, Holland, and France, and to garlic

in Spain and Italy. Bremer (7) in 1934 reported that white rot of onion was widespread in Germany under the name "Flour disease." Among the other countries in which white rot has been reported are Argentina (13), Australia (1), Brazil (6), Egypt (15), and South Africa (9).

According to Walker (29), the first authentic record of white rot in the United States was on onion and garlic from Oregon in 1918. This was followed by a report of the disease on onion in Virginia in 1923. The disease was reported from Kentucky in 1925 (25), New Jersey in 1936 (10, p. 190), and California in 1938 (11). White rot was reported on shallot, onion, and garlic from Louisiana in 1942 (21). However, the disease probably had been present there for some years previous to that date. While the disease has been recorded from a number of different locations in the United States, no extensive infested areas have been found. For this reason no large scale losses have been reported from this country. Small infested areas were found in Louisiana in which the shallot and garlic crops were a total loss. The disease is a potentially serious threat to the growing of onion and garlic and shallot in the State. These crops are all grown during the fall, winter, and spring seasons when temperatures are most favorable for white rot development.

THE CAUSAL FUNGUS

The organism causing the disease was first described by Berkeley (4) in 1841, as a sterile fungus and named *Sclerotium cepivorum*. Voglino (26) studied the organism and renamed it *Sphacelia allii* after he found that definite conidiophores and conidia were produced. Later Walker (29) did not confirm Voglino's description. Cotton and Owen (8) noted the production of microspores which they did not succeed in germinating. The writer has also observed the presence of microspores in old tube cultures on Czapek's agar. Several attempts were made to germinate the microspores without success.

Sclerotia germinate readily under proper environmental conditions. The fungus may live in the soil for several years without the host plants being present. Ogilvie and Hickman (17) reported that onions planted in a field that had been free of onions for at least eight years were severely affected with white rot. Nattrass (16) recommended a ten-year rotation to help control the disease.

Host Range

White rot apparently is confined to plants belonging to the genus *Allium*. Most of the cultivated members of this genus are susceptible to the disease. Walker (29) recorded the following species as being affected: onion (*Allium cepa*), Welsh onion (*Allium fistulosum*), leek (*Allium porrum*), garlic (*Allium sativum*), and shallot (*Allium ascalonicum*). Tims (22) reported the occurrence of white rot on wild onion (*Allium*

canadense). This plant grows wild in many shallot fields in southern Louisiana. Naturally infected plants were found in a shallot field where white rot was prevalent on garlic and shallot. Inoculations with *Sclerotium cepivorum* from shallot showed that the wild onion was susceptible to the disease.

Cultural Studies

Cultures of *Sclerotium cepivorum* were easily obtained by plating sclerotia from diseased shallot plants, or by placing fresh diseased material in a moist chamber and transferring to agar slants the mycelium that developed within 24 hours. A number of strains of the fungus were isolated from diseased onion, garlic, and shallot. Most of these strains varied little in growth characters or pathogenicity. The cultures used in the later tests were all obtained from diseased shallot plants grown in the infected areas around Convent, La.

Variations. Strains of the fungus have been reported that varied in cultural characters as well as in pathogenicity. Walker (29) tested several cultures from various sources and found one culture which produced profuse white mycelium that obscured the sclerotia. He also tested a culture from Brittany that was non-pathogenic on onion. Nattrass (15) reported that a culture from Holland grew more vigorously than one from Egypt, producing a denser more floccose type of mycelium and forming microsclerotia up to 16 days later.

Early in our work two strains of the fungus obtained from shallot varied in certain growth characters, such as rate of growth, numbers of sclerotia, and color of mycelium. These cultures (called A and B) were obtained from separate single sclerotia. Culture A (Fig. 2) produced numerous sclerotia on Czapek's agar with a minimum of mycelium; culture B developed a heavy mat of white mycelium with very few sclerotia. The latter culture also grew more slowly than culture A. Transfers of these two cultures were made at approximately monthly intervals for three years with little apparent change in any of these characters, except that culture B developed a number of sectors. Several inoculation tests were made on shallot with the two cultures. Culture A produced typical white rot symptoms, but culture B caused no injury. Shallot plants from a typical test are shown in figure 3.

The sectors that developed in culture B varied from pure white to a dark smoky gray. The sectors also varied considerably in color of mycelium, rate of growth, and production of sclerotia. Most of them produced few or no sclerotia on Czapek's agar, a medium very favorable for sclerotial development in the normal cultures of *Sclerotium cepivorum*. None of the sectors showed any evidence of parasitism on shallot. Four of the sectors, along with two typical cultures, are shown in figure 4.

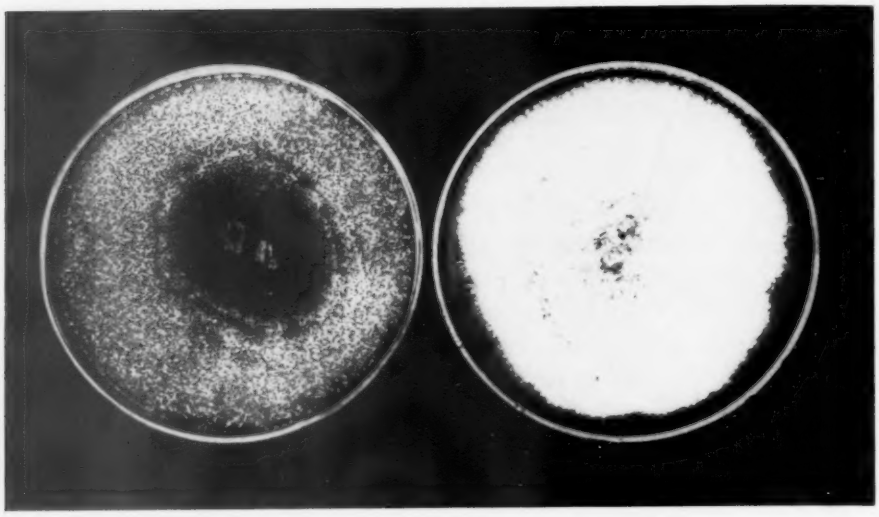


FIG. 2. Cultures of *Sclerotium cepivorum* on Czapek's agar. Culture A on left has typical sclerotial development, while culture B on right has white mycelium with few sclerotia.

FIG. 3. Shallots inoculated with cultures of *Sclerotium cepivorum*. Culture A on left, B on right.

pH relationships. Certain aspects of the disease in Louisiana indicated that white rot might be very restricted in its development in certain soils. It apparently has been present in rather limited areas for a good many years, although no special effort has been made to prevent its spread. Shallots, onions, and garlic are grown regularly in these areas under conditions apparently ideal for the disease to develop and spread from field to field. This failure to spread more extensively seemed to indicate that the causal fungus might be sensitive to comparatively slight differences in soil environment. Most of the white rot infested soils were found to be rather acid (pH 5 to 6.3). Early cultural studies had also indicated that

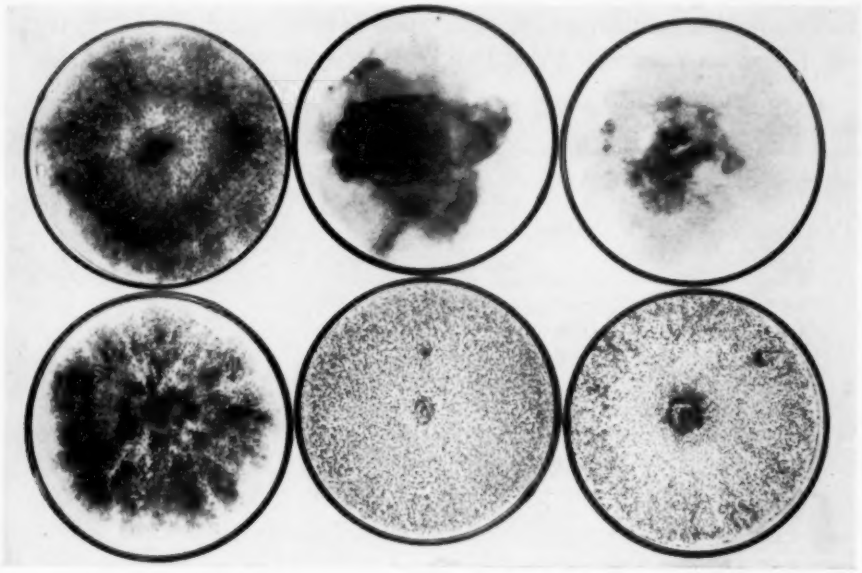


FIG. 4. Cultures of Sclerotium cepivorum. The two plates on lower right show typical sclerotial forms; the others are sectors from culture B.

the fungus was favored by an acid medium. For these reasons a number of tests were conducted in liquid and on solid media to determine the pH relationships of *Sclerotium cepivorum.*

Bacto bean agar was used for a series of pH tests. A pH series ranging from 3.1 to 10.1 was prepared by adding different amounts of N/5 HCl or N/5 KOH to the agar after sterilization. Four plates were poured immediately from each of two flasks at each pH concentration, six of them being inoculated with a suitable culture of the fungus and the other two left as controls. The pH of the agar that remained in the flasks was determined as soon as the plates were poured. The plates were incubated at 22°-23° C. for ten days, after which the agar in all the plates was remelted and pH determinations were made.

The results of a typical test are in table 1. The pH of the nonin-

oculated plates remained relatively stable except in the high alkaline series. But the agar in all the inoculated lots became more acid except in the two most acid series. The fungus growth was good in all plates except those with an initial pH of 9.6 to 10.

Preliminary tests with Richard's solution[1] indicated that the white rot fungus might be definitely limited in growth above pH 7. Several series of tests were made to determine the pH limits for growth of the fungus in this medium. The method of Karrer and Webb (12) was used for determining the amounts of acid or alkali necessary to produce the desired pH range. A series was prepared in which the pH varied from 1.8 to 7.7. The necessary amounts of N/5 HCl or N/20 KOH to bring about the desired concentration were added after sterilization. Six flasks were used at each pH concentration. Four flasks in each lot were inoculated with a

TABLE 1.—*Relation of pH to growth of Sclerotium cepivorum on Bacto bean agar plates*

Initial pH	Final pH		Growth of the fungus
	Control	Inoculated	
3.2	3.1	3.1	Good
3.5	3.5	3.2	Good
4.0	4.1	3.3	Good
5.1	5.2	3.5	Good
7.1	6.7	3.6	Good
8.8	8.4	3.8	Good
9.6	8.9	4.4	Fair
10.1	9.0	6.8	Slight

small disk cut from an agar plate of a rapidly growing culture of *Sclerotium cepivorum*. The other two flasks were kept as controls and the medium was tested for changes in pH at the end of the experiment. The flasks were incubated at 22°-23° C. for 21 days, after which the necessary observations were made and further pH determinations were obtained. The results are in table 2.

Growth of the fungus was definitely limited between pH 2 and 7. There was little change in the pH of the controls, and not much change in the inoculated flasks except at initial pH of 6.2 and 6.3. The fungus grew best and produced sclerotia most abundantly at pH 6.3.

Since the growth of the fungus seemed to be definitely limited below pH 2 and above pH 7 in Richard's solution, further attempts were made

[1] Richard's solution:

KH_2PO_4	0.5 gm.
KNO_3	4.0 gm.
$MgSO_4 + 7\ H_2O$	0.5 gm.
NH_4NO_3	10.0 gm.
$FeSO_4$	Trace
Sucrose	30.0 gm.
Distilled H_2O to make 1 liter.	

to determine the pH limits more accurately. In a similar test, there was no growth at pH 1.9 and 7.1, traces of growth at pH 2.1 and 6.9, slight growth at pH 6.8, fair growth at pH 2.3, and excellent growth at pH 6.4.

Similar tests were conducted with Czapek's solution[2]. A series of flasks was set up with pH values ranging from 2.1 to 9.8. All of the flasks were inoculated and incubated at 23°-24° C. for 21 days. One set of inoculated flasks was tested after 7 days, another after 16 days, and the remainder after 21 days. The results in table 3 show that slight changes in pH level occurred after 7 days, but the most important changes took place before the 16th day. The fungus grew well and produced numerous sclerotia at initial pH levels from 5.4 to 7.2. But there was some growth from pH 2.7 to 9.1. Shubbs[3], working in Australia, obtained similar results with *Sclerotium cepivorum* in a beefine-peptone-dextrose medium.

TABLE 2.—*Relation of pH to growth of the white rot fungus in Richard's solution*

Initial pH	pH after 21 days		Growth of the fungus
	Control	Inoculated	
1.8	1.7	1.7	None
1.9	1.8	1.8	None
2.1	2.1	2.1	None
2.3	2.3	2.2	Moderate
6.2	6.2	4.4	Good
6.3	6.4	4.5	Excellent
7.2	6.7	6.7	None
7.5	6.9	6.9	None
7.7	6.8	7.0	None

These limited tests with several media indicated that *Sclerotium cepivorum* grew over a wide pH range on certain media, but was definitely limited on other media such as Richard's solution. Since the white rot fungus would grow on media with such a wide pH range, field control by changing the pH of the soil seemed questionable. However, tests were conducted in which lime was applied to infected soil in an effort to control the disease.

[2] Czapek's solution:

$MgSO_4 + 7\ H_2O$	0.5 gm.
KH_2PO_4	1.0 gm.
KCl	0.5 gm.
$FeSO_4$	Trace
$NaNO_3$	2.0 gm.
Sucrose	30.0 gm.
Distilled H_2O to make 1 liter.	

[3] Personal communication.

CONTROL

Apparently no completely satisfactory method has been developed for the control of white rot on any of its hosts. Most of the control experiments have been reported on onion from Great Britain. Very little work has been reported on control of the disease in shallots. Asthana (3) reported one test in which lime was applied to the soil, but the results were inconclusive. Somewhat later Tims (24) mentioned that the addition of lime and other chemicals including Semesan and mercuric chloride to the soil reduced the amount of white rot in shallots.

Numerous onion varietal resistance trials have been reported from the British Isles. In 1920 Cotton and Owen (8) reported that shallot and leek apparently were more resistant than onion. However, all the evidence obtained in Louisiana indicates that the shallot is rather susceptible. Nattrass (16) reported onion varietal tests in infested soil. There was some

TABLE 3.—*Relation of pH to the growth of the white rot fungus in Czapek's solution*

Initial pH	pH of inoculated flasks after			Growth
	7 days	16 days	21 days	
2.2	2.1	2.1	2.3	None
2.7	2.8	3.6	4.5	Fair, few sclerotia
5.4	3.9	4.4	4.6	Good, numerous sclerotia
6.5	6.5	4.8	4.5	Good, numerous sclerotia
7.2	7.2	5.0	4.8	Good, numerous sclerotia
8.1	7.9	7.2	6.6	Fair, few sclerotia
9.1	8.2	7.4	7.4	Slight
9.5	8.5	8.5	8.4	Trace
9.8	8.7	8.7	8.6	None

variation in percentages of diseased plants but no evidence of a satisfactory degree of resistance. In 1930 Smith (19) reported that "a satisfactory degree of resistance to white rot has been shown by the 'white welch' onion variety in Lancashire and Cheshire." Later reports (2) indicated that onion varieties with marked resistance to white rot have been developed as a result of several years' tests at Manchester University. In 1941 Ogilvie and Walton (18) listed several onion varieties that had moderate resistance to white rot.

The use of chemicals for control of white rot of onion has also been reported. In 1930 Wingard (32) treated onion bulbs with formaldehyde before planting them in infested soil. This treatment gave no control. Ogilvie and Hickman (17), working in England, reduced the white rot in diseased soil by the application of hydroxymecuri-chlorophenol at the rate of

1 oz. per sq. yd. of soil, but they did not obtain satisfactory control. Later Booer (5) reported promising results from England where he applied mercurous chloride dust to the drill with the onion seed. One pound of the 4 per cent dust to 25 yards of row gave 7.9 tons of onions per acre compared to 1.58 tons on untreated rows. Rotation has been recommended for white rot control, but few data have been reported to show the effectiveness of such measures. Shubbs[4] in Australia mentioned "two cases where market gardeners have eliminated losses due to white rot disease by the liberal use of lime, rotation and clean cultivation." Asthana (3) found that comparatively large amounts of potassium sulphate reduced the amount of disease in onions; and lime applied at the rate of 2-5 tons per acre also reduced the incidence of white rot.

All of the experiments for the control of white rot of shallot were conducted on a small block of naturally infested soil near Convent, La. The block consisted of 6 rows six feet wide and 350 feet long. The plot was heavily infested with the white rot fungus for about half its length while the other end was more lightly infested. A crop of garlic grown in this block of land in 1942 was almost totally destroyed by the disease. Shallots were planted in this plot in 1943, 1944, and 1945. A portion of the block was used in the lime tests during the three-year period. Parts of the remainder were used for miscellaneous tests. The common white shallot was used in all the tests unless otherwise indicated. Most of the plantings were made in October and the shallots were harvested the following December or January.

APPLICATION OF LIME TO THE SOIL

In 1943, the most heavily infested end of the plot was divided into 12 sections consisting of 6 rows 6 feet wide and 25 feet long. A 5-foot buffer was arranged between each two plots. Four replications of three different treatments were used as follows: A, hydrated lime applied Sept. 15 at the rate of 1,000 lb. per acre; B, lime at 1,500 lb. per acre; and C, check, no treatment. Soil samples were taken from each plot before the lime was broadcast over the plots, and at intervals later. The shallots were planted October 7. The data are in table 4.

White rot first appeared December 5 and was well developed by December 20 when the disease counts were made. At this time the shallots were ready to pull for market. The healthy bunches indicated in table 4 were suitable for market. The shallots were left in the field ten days longer and at this time most of the bunches were diseased. The data show that the lime reduced the amount of early infection. But a few days after maturity the plants in the treated plots were almost as severely affected as those in the untreated plots.

[4] Personal communication.

TABLE 4.—*The effect of lime on the pH of infested soil, and on the amount of disease in shallots, in 1943*

Amount of lime added to the soil	pH of soil[a]		Total no. of bunches in 4 plots	Number of healthy bunches	Percentage of healthy bunches[b]
	Before lime	After lime			
1000 lb.	5.7	6.4	427	290	68
1500 lb.	5.7	6.6	416	308	74
None	5.7	5.7	414	240	58

[a] Tests were made 30 days after application of the lime.

[b] The counts were made Dec. 20, when the shallots were ready to pull.

The same block of infested soil was used in 1944-1945. Additional lime was applied at the rate of 1,500 lb. per acre to two of the lime plots of the previous year as shown in table 5. The lime was applied Sept. 21, and the shallots were planted Oct. 2, 1944. The disease counts and pH readings were made Jan. 3, 1945. The diseased plants were judged by the above ground symptoms alone.[5]

TABLE 5.—*Effect of lime on the pH of infested soil and on the amount of white rot in shallots. Data obtained Jan. 3, 1945*

Section	Amount of lime added to soil		pH of soil	Total no. bunches produced	No. of dead bunches	Percentage of bunches diseased
	1943	1944				
1	1000 lb.	None	5.9	161	4	20
2	None	None	5.5	145	2	29
3	1500 lb.	1500 lb.	7.1	158	0	6[a]
4	1000 lb.	None	5.9	152	2	23
5	None	None	5.4	154	2	22
6	1500 lb.	1500 lb.	7.2	145	0	5[a]
7	1000 lb.	None	6.0	156	3	20

[a] Plots 3 and 6 were in far better condition than the others. Most of the bunches could have been pulled in good condition.

The data in table 5 show that the additional amounts of lime reduced white rot in its early stages. A fairly satisfactory crop of shallots could have been obtained from badly infested soil if the plants had been pulled at the right time. The shallots in this test were left in the field an additional 12 days to see whether or not the lime would keep the disease under control after the plants matured.

The shallots were all pulled and examined Jan. 15. At this time more of the shallots in all the plots were diseased. However, 50 per cent of the bunches in plots 3 and 6 were in a marketable condition, while only 10 to 15 per cent of the plants in the adjoining plots were in good condition.

[5] When the plants were pulled, washed, and examined carefully some of the shallots without above ground symptoms were found to be diseased.

This test indicated that the addition of lime sufficient to bring the pH of the soil to about 7 reduced the amount of white rot considerably in the earlier stages of the disease. After the shallots matured sufficiently for the market the leaves died back quickly and the disease made rapid inroads on the weakened plants.

The additional lime applied in 1944 brought the pH of the soil to approximately the neutral point, as is shown in table 5. The plots that received small amounts of lime in 1943 showed little residual effect on the amount of disease in the 1944–1945 crop.

Some of the plots in the block of land used for the liming tests in the 1943 and 1944–45 seasons were used in the 1945–46 season for other purposes. But some information was obtained on the carry-over effects of the lime applied during the two previous seasons. Shallots were planted somewhat later than in the other tests (Oct. 24, 1945). The plants grew more slowly than usual and the white rot showed up later than in any previous season. The shallots were pulled and examined Feb. 13, 1946. At this time the plants had passed their peak of development and had begun to deteriorate somewhat. From plot 1, 12 per cent of the plants were healthy, from plot 3, 30 per cent, and from plot 5, 38 per cent. These figures show little residual effect of the previous applications of lime.

ADDITION OF CHEMICALS TO THE SOIL

Several tests were conducted in which chemicals were applied to infested soil where shallots were planted. Walker (31) found that 138 ml. of mercuric chloride (1–750, 1–1500) per plant applied at transplanting time greatly reduced the incidence of cabbage club root. In our tests the chemical was applied to the soil around the growing plants. A number of seed treatment chemicals were also applied to shallot sets before they were planted in diseased soil.

In the fall of 1943 a preliminary test was conducted in which formaldehyde and Semesan were applied around shallots growing in diseased soil. Formalin (40 per cent commercial) was used in two strengths, 30 and 120 ml. per gallon of water, and Semesan 30 gm. to 1.5 gal. Small plots consisting of single rows 25 feet long were replicated 4 times. The shallots were from 4 to 7 inches tall when the treatments were applied Oct. 7. A small amount of the chemical was poured around each plant, wetting an area 3-4 inches in diameter and about the same depth. The chemical was used at the rate of 1.5 gal. per 100 feet of row. Dry soil was pulled up around the plant to prevent rapid evaporation of the formaldehyde. The formaldehyde caused a severe yellowing of the plants, but none of them was killed. The Semesan caused no apparent injury.

The plants were examined Dec. 20 when they were ready to pull for market. At this time the average percentage white rot for each treatment was as follows: Semesan, 7 per cent; formaldehyde 30 cc. per gal., 27 per

cent, formaldehyde 120 cc. per gal., 16 per cent; and untreated plots, 41 per cent. Two weeks later most of the plants were severely diseased, but in the Semesan plots most of the shallots were in good condition.

In October, 1944, the soil treatment tests were continued in the same plot. Different portions of the plot were used each time in order to prevent after-effects of previous soil treatments. Shallot plants one week old were used in this test. The plants were several inches high and were just beginning to divide when the chemicals were applied (Oct. 9). Semesan (1 oz. per 1 gal. water) and mercuric chloride (1–500) were used at the rate of 80 ml. per plant. An area about 5 inches in diameter and 3-4 inches deep was moistened around each plant. Small replicated plots were used with appropriate controls. The plants were pulled and examined Jan. 15, 1945.

TABLE 6.—*Effect of chemicals applied to soil on white rot of shallot in 1944-1945*

Treatment	Total no. bunches produced	Number of bunches diseased	Number of bunches dead
Semesan, 1 oz. per gal.	138	2	0
$HgCl_2$, 1-500	137	0	0
Untreated	140	96[a]	36

[a] This figure includes dead bunches.

The data in table 6 show that both chemicals gave almost complete control of white rot under the conditions of this test.

In the fall of 1945, a soil treatment test was made on a slightly larger scale than the previous ones. Mercuric chloride (1–1000) was applied around young shallot plants at the rate of 80 ml. per plant. One block of 6 rows 28 ft. long was treated. The sections on each end of the treated block were left untreated. The chemical was applied Nov. 15, when the plants were 5-7 inches tall. All the plants were pulled and examined for white rot Feb. 13, 1946. The data are in table 7. The mercuric chloride at 1–1000 strength did not give as good control as the 1–500 strength used previously. However, most of the plants in the treated plots held up considerably longer than those in the untreated plots.

TABLE 7.—*Effect of mercuric chloride applied to the soil on white rot of shallot in 1945-46*

Treatment	Total no. of bunches	Number of bunches diseased on		
		Jan. 8	Jan. 30[a]	Feb. 13
$HgCl_2$, 1-1000	325	10	50	64
Untreated	331	72	128	184

[a] Plants ready to pull at this time.

Preliminary tests were made in 1944 with D-D (1, 3-dichloropropene-1,2-dichloropropane) for control of white rot. The D-D was applied to the soil in three concentrations: (1) 5 ml. in holes 6 inches apart; (2) 5 ml. 1 ft. apart; and (3) 15 ml. 1 ft. apart. The holes were 1 inch in diameter and 4-5 inches deep. The D-D was placed in the holes by means of a rubber tube, and the holes were filled with soil immediately. The treatments were applied in small replicated plots. Shallot transplants were set out two weeks after the D-D was applied. Treatments 1 and 2 decreased the amount of white rot appreciably but did not give satisfactory control.

Treatment of seed sets. In the fall of 1944 a small test was made with fungicidal dusts on shallot sets. Barbak D (mercuric phenyl cyanamide) and Arasan (50 per cent tetramethyl thiuramdisulphide) were used. The sets were shaken in paper bags with an excess of the dust, then planted in infested soil. In this test there was no appreciable difference in the amount of disease in the treated and untreated plots. The following year (1945) Arasan was used in a slightly different way. The shallot sets were moistened with water and then coated with Arasan dust before being planted in diseased soil. Again the Arasan failed to give any appreciable control of white rot.

VARIETAL REACTION TO WHITE ROT

Two white shallot varieties make up the bulk of the crop in Louisiana. The common or Chac Bay variety is a small pin-leaf type that has been grown in the State for many years, and the Louisiana Pearl is a large flat leaf type that was bred at the Louisiana Agricultural Experiment Station several years ago for resistance to pink root (24). There are also two red varieties grown in limited areas.

The four shallot varieties or types were grown in infested soil to determine whether there might be some resistance to the disease in any of them. Several shallot seedlings of varying types bred at the Experiment Station in Baton Rouge were also tested for resistance. None of them showed any evidence of resistance to the disease.

DISCUSSION

Walker (30) has shown that white rot of onion is definitely favored by comparatively low soil moisture. He found that the greatest development of the disease occurred when the soil moisture was kept at about 40 per cent of the water-holding capacity. Field observations made in Louisiana show clearly that white rot is much more severe in the higher, better drained portions of the field than in lower, wetter areas. The plants on the ditch bank rows where drainage is best are invariably the most severely affected. In some of the soil treatment tests, the disease was completely inhibited by the treatments in areas 12-15 ft. from the drainage ditch, while on the ditch bank row there was some injury. Factors such as

soil aeration and differences in the physical condition of the soil also may affect the development of the disease.

The matter of dissemination of white rot in this State has been of some interest. One grower whose small farm is most severely affected states that he has had the disease on his place for at least 20 years. No apparent effort has been made to prevent the spread of the disease, but the amount of spread has been remarkably small considering the possible means of dissemination. The writer has been working on one plot of heavily infested soil for four seasons. This plot is separated from an adjoining farm by a head land only 12 ft. wide. Shallots and garlic are grown regularly on the next farm but no white rot has been observed there.

The principal means of dissemination of white rot in shallots here appear to be (1) shallot transplants from diseased areas and (2) the transfer of infested soil by the use of farm implements, on the shoes of laborers, or on the feet of work animals. The disease is not carried through on infested shallot sets to any appreciable extent because they almost invariably rot during the summer. The writer has collected many slightly infected shallot sets for experimental use, but they invariably rotted before they could be used. However, garlic is grown on some shallot farms. Slightly infected garlic bulbs might transfer the causal fungus to new areas.

White rot has proved to be a very difficult disease to control in Louisiana. The addition of lime in sufficient quantity to bring the soil to about pH 7 reduced the amount of disease appreciably but did not give satisfactory control. One year after the lime was applied the disease was almost as severe as before. The use of lime had the same effect as most of the control measures — it only delayed the development of the disease. If the shallots were pulled at exactly the right time the lime, Semesan, and mercuric chloride greatly reduced the severity of the disease. The 1944-45 tests with Semesan and mercuric chloride gave almost complete control because they delayed the development of white rot long enough for the shallots to be harvested. But in most of the treatment tests the plants were killed if they were left in the field very long after maturity.

None of the shallot varieties being grown in the State have any appreciable degree of resistance. However, some shallot breeding is being done for control of other diseases. These progenies will be tested for possible resistance to white rot.

The most promising method for controlling white rot of shallot in Louisiana seems to be early planting. Most of the shallots are planted in September or October. These plants are usually harvested in December or early January. Other plantings are made later during the winter. Often as many as three crops of shallots may be grown on the same soil during one season, beginning about September 1 and lasting until May 1. White rot has not been observed here earlier than about December 1. If

the severely infested areas are planted in late August or early September the shallot crop can be harvested in October or November before the white rot develops to any appreciable extent. Thus one good crop might be harvested from diseased soil each year.

SUMMARY

White rot of shallot has been known in Louisiana only a few years. At present the infested areas are rather small, but the disease is a potential threat to the entire shallot growing industry in the State.

The disease shows as a wilting, yellowing, and dying of the leaves associated with rotting of the roots and basal portions of the shallot plants. Grayish white mycelium and small black sclerotia develop on the underground portions of the affected plants.

White rot is a cool weather disease and develops in Louisiana only during the winter months from December through March. Shallots are grown from September into May, thus exposing them to the disease during a considerable portion of the time that they are in the fields.

The disease is known to affect most of the cultivated species of *Allium*.

Strains of *Sclerotium cepivorum* from shallot were found that varied considerably in color and amounts of mycelium, numbers of sclerotia, and pathogenicity on shallot.

The fungus grows well over a rather wide pH range on media such as Bacto bean agar and Czapek's solution. However, in Richard's solution growth was sharply limited between pH 2 and 7.

Lime applied at the rate of 1,500 lb. per acre reduced the severity of white rot in shallot, but did not give satisfactory control. Semesan and mercuric chloride applied to the soil around growing shallot plants gave fairly good control.

Seed set treatments with a number of fungicidal dusts were ineffective in reducing the amount of disease.

None of the shallot varieties now being grown in the State have any appreciable resistance to white rot.

Early planting of shallots is probably the best means of control in Louisiana. If they are planted early enough (Aug. 20 to Sept. 15), one good crop of shallots can be harvested before white rot develops.

LOUISIANA AGRICULTURAL EXPERIMENT STATION,
BATON ROUGE, LOUISIANA.

LITERATURE CITED

1. ANONYMOUS. Plant Diseases. Notes contributed by the Biological Branch Agr. Gaz. N. South Wales 49: 423–427. 1938.
2. ANONYMOUS. Reports on the work of Agricultural Research Institutes and on certain other agricultural investigations in the United Kingdom. 1930–31. 377 pp. London. 1932 (Abstr. in Rev. Appl. Myc. 12: 139–140. 1933.).
3. ASTHANA, R. P. The influence of chemical manures upon "white rot" of Allium. Proc. Indian Acad. Sci. Sec. B 22: 168–174. 1945.

4. BERKELEY, M. J. Notices of British fungi. Ann. and Mag. Nat. Hist. 6: 355–365. 1841.
5. BOOER, J. A. Experiments on the control of white rot (*Sclerotium cepivorum* Berk.) in onions. Ann. Appl. Biol. 32: 210–213. 1945.
6. BRANDÃO, J. S. Algumas doencas do Alho. Reprinted from Bol. Minist. Agric., Rio de Janeiro. 1942. 6 pp. 1942. (Abstr. in Rev. Appl. Myc. 22: 509. 1943).
7. BREMER, H. Die Mehlkrankheit der Zwiebeln. Nachrichtenbl. Deutsch. Pflanzenschutzdienst 14: 37–38. 1934. (Abstr. in Rev. Appl. Myc. 13: 613. 1934.)
8. COTTON, H. D., and M. N. OWEN. The white rot disease of onion bulbs. Jour. Min. Agr. Great Britain 26: 1093–1099. 1920.
9. DUPLESSUS, S. J. White mold in onions. Farming in South Africa 7: 112–114. 1932.
10. EDSON, H. A. Diseases of plants in the United States in 1935. U. S. Dept. Agr. Plant Dis. Rptr. Suppl. 96. 1936.
11. GARDNER, M. W. *Sclerotium cepivorum* found on garlic in California. U. S. Dept. Agr., Plant Dis. Rptr. 23: 36. 1939.
12. KARRER, J. L., and R. W. WEBB. Titration curves of certain liquid culture media. Ann. Mo. Bot. Gard. 7: 299–305. 1920.
13. MARCHIONATTO, J. B. Notas sobre algunos Sclerotium parasitos de las plantes economicas. Physis (Rev. Soc. Argentina Cien. Nat.) 11: 301–305. 1933. (Abstr. in Rev. Appl. Myc. 13: 327. 1934).
14. NATTRASS, R. M. Onion immunity trials. 1927. Ann. Rpt. Agr. and Hort. Res. Sta. Long Ashton, Bristol for 1927. p. 106. 1928.
15. ———. The occurrence of the "white rot" of the onion (*Sclerotium cepivorum* Berk.) in Egypt. Min. Agr. Egypt. Tech. and Sci. Service (Plant Protection Sect. T), Bull. 107. 1931.
16. ———. The white rot of onions in Cyprus. Cyprus Agri. Jour. 28: 98–100. 1933.
17. OGILVIE, L., and C. J. HICKMAN. Progress Report on vegetable diseases. Rept. Hort. Res. Sta. Long Ashton, Bristol, for 1937. pp. 96–109. 1938.
18. ———, and C. L. WALTON. Diseases and pests of onions and leeks. Wores. Agri. Quart. Chron. 9: 57–65. 1941.
19. SMITH, E. HOLMES. Autumn sowing variety of onion resistant to frost and white rot. Gard. Chron. 86: 429–430. 1929.
20. TIEBOUT, G. L., and JOSEPH MONTELARO. Louisiana Shallots. La. State Univ. Agr. Ext. Circ. 260. 1946.
21. TIMS, E. C. White rot of Allium in Louisiana. U. S. Dept. Agr., Plant Dis. Rptr. 26: 219–221. 1942.
22. ———. Further observations on white rot of Allium in Louisiana. U. S. Dept. Agr., Plant Dis. Rptr. 27: 280–281. 1943.
23. ———. Diseases of onion, shallot, and garlic in Louisiana during the 1942–43 season. U. S. Dept. Agr., Plant Dis. Rptr. 27: 281–283. 1943.
24. ———. White rot of shallot and its control. (Abstr.) Phytopath. 37: 437. 1947.
25. VALLEAU, W. D. White rot (*Sclerotium cepivorum*) of onion reported from Kentucky. U. S. Dept. Agr., Plant Dis. Rptr. 9: 46–47. 1925.
26. VOGLINO, PIETRO. Sul parassitismo e lo sviluppo dello Sclerotium cepivorum Berk. Nell' allium sativum L. Stazioni Sperimentali agrariae Italiane 36: 89–106. 1903.
27. WALKER, J. C. Occurrence of white rot of allium (*Sclerotium cepivorum* Berk.) in Europe and America. (Abstr.) Phytopath. 14: 26. 1924.
28. ———. Observations on the cultivation and diseases of cabbage and onions in Europe, 1922. U. S. Dept. Agr., Plant Dis. Rptr. Suppl. 32. 1924.
29. ———. White rot of Allium in Europe and America. Phytopath. 14: 315–322. 1924.
30. ———. The influence of soil temperature and soil moisture upon white rot of Allium. Phytopath. 16: 697–710. 1926.
31. ———, M. A. STAHMANN, and D. E. PRYOR. Efficacy of fungicidal transplanting liquids for control of clubroot of cabbage. Phytopath. 34: 185–195. 1944.
32. WINGARD, S. A. White rot of onions in Virginia. U. S. Dept. Agr., Plant Dis. Rptr. 14: 104–105. 1930.

INFECTIOUS CHLOROSIS OF PHENAX SONNERATII

KARL M. SILBERSCHMIDT[1]

(Accepted for publication January 20, 1948)

During a survey of species of Malvaceae, attacked by "Infectious Chlorosis," we found a weed, exhibiting similar chlorotic spots, but which belongs to the species *Phenax Sonneratii* (Poir.) Wedd. of the Urticaceae.[2] The first natural habitat of this diseased weed observed by us (on February 3, 1946) was a sandy, shaded place called "Prainha" near the beach of São Vicente (State of São Paulo). Later (April 14, 1947) we collected specimens of *Phenax Sonneratii* exhibiting the same type of symptoms at another sandy spot of the beach of Santos called "Bocaina." On both occasions the leaves of the attacked plants displayed a pronounced veinclearing (Fig. 1, a and b), light green or yellowish angular spots limited largely by the secondary veins (Fig. 1, a).

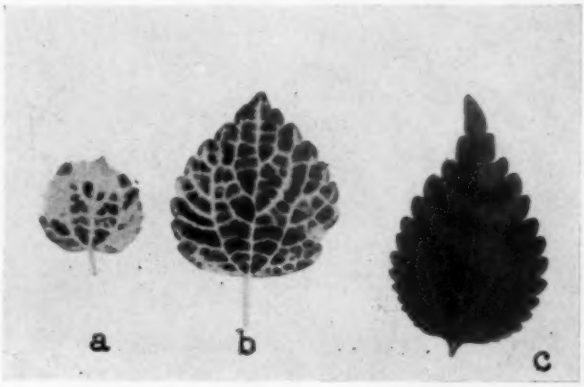


FIG. 1. Leaves of *Phenax Sonneratii*, (a and b) from a plant naturally infected with "Infectious Chlorosis," and (c) from a healthy plant.

In connection with our studies on Infectious Chlorosis of the Malvaceae (9) it was interesting for us to determine if a graft-transmissible disease characterized by chlorotic symptoms could be found also on representatives of the Urticaceae. We brought rooted, diseased plants of *Phenax* from Santos to our Institute in São Paulo, where we grew them in our greenhouse. With this diseased material from the two natural sources we performed the following experiments on the transmissibility of the disease.

On April 1, 1947, four healthy plants of *Phenax* were grafted with diseased scions of the same species. Eighteen days later two of the scions had taken and the respective stocks displayed on their young leaves

[1] Chefe da Seção de Fisiologia Vegetal, Instituto Biológico, São Paulo, Brasil.

[2] We are indebted for the classification to Mr. J. F. Toledo of the "Departamento de Botânica do Estado," São Paulo, Brasil.

chlorotic symptoms similar to those shown on the scion. Diseased leaves were confined almost entirely to the grafted shoots; other shoots of the same plants continued producing normal green leaves for months.

Other positive results in our attempts to transmit the disease by grafting to healthy plants of the same species were obtained in our experiments of July 7 and August 11. Six out of 18 healthy plants of *Phenax Sonneratii* grafted with chlorotic scions contracted the disease. Healthy test

FIG. 2. Plant of *Phenax Sonneratii*, (a) experimentally infected with "Infectious Chlorosis," (b) a healthy plant of the same species.

plants, grown on the same bench as the grafted ones, and the control plants, which had been grafted with scions of healthy plants or with healthy shoots of diseased plants, never displayed symptoms. Figure 2 shows one of the stocks infected by grafting with "Infectious Chlorosis" beside a healthy control plant.

We did not succeed in transmitting the disease by grafting to *Boehmeria nivea* (Urticaceae) or to *Sida rhombifolia* (Malvaceae).

The seeds collected from diseased sources produced plants without any symptoms of the chlorotic disease (June 3, 1947).

On September 8, we inoculated 7 healthy plants of *Phenax Sonneratii* with sap of diseased shoots, and we rubbed 5 other plants with sap of healthy plants. Until now all these plants have remained without any symptoms of the chlorotic disease.

Returning now to our experiments on the transmission of the disease by grafting, we wished to ascertain if the progress of the disease along a grafted shoot could be speeded up experimentally. Following the method conceived by Hildebrand (6) and used efficiently by Cochran (4) we covered the healthy tip of the grafted shoots with black pasteboard. Taking off the covers after a fortnight, we observed that many of the older shaded leaves had dropped, but that the young, developing leaves displayed symptoms of the disease. The tips of grafted plants, which had not been covered with black pasteboard, remained healthy for a long time.

In view of the characteristic symptoms of this disease and of its transmissibility only by grafting, we consider it a virus disease and suggest it be included in the group of "Infectious Chloroses."

In the literature—as far as we could verify—few virus diseases among representatives of the Urticaceae have been described. As early as 1929 McKinney (7) had observed *Fleurya podocarpa* Wedd. (Urticaceae) displaying yellow mosaic in West Africa. Later, Severin (8) cited *Urtica urens* as host plant of experimentally transmitted sugar-beet curly top. Gardner and Whipple (5) mentioned "nettle" among the experimental host plants of the spotted wilt of tomato. More recently Blattny, Robek, Stary, and Ryzkov in two papers (2, 3) have described a sap-transmissible mosaic disease of *Urtica dioica* and *Urtica urens*, probably the same disease which Blattny (1) had observed much earlier on naturally infected plants of *Urtica dioica*. In none of those publications, however, do the authors refer to a disease of the Urticaceae of the general character of "Infectious Chlorosis" transmissible by grafting, but not by sap inoculation. Therefore, we consider *Phenax Sonneratii* a new host plant of "Infectious Chlorosis."

INSTITUTO BIOLÓGICO,
SÃO PAULO, BRASIL.

LITERATURE CITED

1. BLATTNÝ, C. Vertikální rozšíření virových chorob. Ochrana Rostlin **13**: 145. 1933. (Ref. in Jour. Agr. Univ. Puerto Rico **19**: 141. 1935.)
2. ———, A. ROBEK, and B. STARY. Lalocnatost listu chmele. Ochrana Rostlin **17**: 67–69. 1941. (Abstr. in Biol. Abstr. **21**: 1234. 1947.)
3. ———, ———, ———, and N. RYŽKOV. Lze zjistiti napadení kadeřavostí již u chmelových sazeček. Ochrana Rostlin **16**: 54–58. 1940. (Abstr. in Biol. Abstr. **21**: 1234. 1947.)

4. COCHRAN, G. W. The effect of shading techniques on transmission of tobacco-mosaic virus through dodder. (Abstr.) Phytopath. **36**: 396. 1946.
5. GARDNER, M. W., and O. C. WHIPPLE. Spotted wilt of tomatoes and its transmission by thrips. (Abstr.) Phytopath. **24**: 1136. 1934.
6. HILDEBRAND, E. M. Techniques for localizing viruses and intensification of disease symptoms. (Abstr.) Phytopath. **33**: 18. 1943.
7. MCKINNEY, H. H. Mosaic diseases in the Canary Islands, West Africa, and Gibraltar. Jour. Agr. Res. [U.S.] **39**: 557–578. 1929.
8. SEVERIN, H. H. P. Weed host range and overwintering of curly-top virus. Hilgardia **8**: 263–277. 1934.
9. SILBERSCHMIDT, K. Estudos sôbre a transmissão experimental da "Clorose Infecciosa" das Malváceas. Arquiv. do Inst. Biol. **14**: 105–156. 1943.

FUSARIUM WILT OF GARDEN STOCK (MATHIOLA INCANA)

KENNETH F. BAKER

(Accepted for publication January 24, 1948)

An important vascular fusarium disease was observed in seed fields of garden stock (*Mathiola incana* R. Br.) and Ten Weeks stock (*M. incana* var. *annua* Voss) in Santa Barbara County, California, during September, 1946. Apparently there has been no published record of a fusarium wilt of this host, but O. A. Plunkett has informed the writer that he observed a similar disease that killed practically all of the plants in a Los Angeles garden about 1928.

Infected plants of the maturing seed crop have characteristic symptoms. The color of the siliques and stems is faded from the usual grayish tan to a light tawny shade (Fig. 1). All or part of the siliques on a stem are flat, as compared with the plump normal condition, and are found to contain flat, undeveloped seeds. There is a somewhat premature shedding of the leaves progressively from the base upward. Any of these symptoms may develop unilaterally. The plants may be stunted and are sometimes killed. Although symptoms at the time of seed maturation are not conspicuous because of normal loss of leaves and green color, it is easy to recognize infected plants once the differences in appearance are noted. There is commonly a brown discoloration of the vascular elements in roots and stems of affected plants. Root cortex apparently does not decay until the plants are dead.

Young plants have conspicuous symptoms within 30 to 40 days after being transplanted into heavily inoculated soil held at about 21°–24° C. Some of the seedlings quickly wilt and die. Older plants may have a striking vein clearing of basal leaves (Fig. 2); this effect is evidenced progressively upward on the stem. Leaves finally turn yellow, but wither before being shed instead of rapidly abscising as in cabbage fusarium yellows. As the plants mature the field symptoms already described appear.

There are several diseases of stock that may be confused with fusarium wilt. Bacterial blight, a vascular wilt disease induced by *Xanthomonas incanae* (Kend. and Baker) Starr *et al.*, causes a characteristic blackening of the leaf scars of the stem (3) not found with the fungus disease, and does not have the tawny color and vein clearing of the latter. Rhizoctonia foot rot, caused by *Rhizoctonia solani* Kühn, girdles the stems of young plants at soil level and causes a gradual or rapid wilting without the tawny color or leaf vein clearing (1); coarse mycelium can be seen with a hand lens on the surface of the stem lesions. Sclerotinia white blight, caused by *Sclerotinia sclerotiorum* (Lib.) Schroet. occurs commonly in seed fields, producing on the flower stalks localized white lesions which girdle the stems; typical large black sclerotia occur in the pith of such stems. Phyto-

phthora foot rot, caused by *Phytophthora cryptogea* Peth. and Laff. and perhaps other parasitic Phycomycetes, decays the roots and crown and results in a wilting of the top; this disease is easily recognized by the

Fig. 1. Ten Weeks stock plants grown in pots in the greenhouse. Plant on left infected with fusarium wilt, showing general stunting and leaf killing, with flattened and light tawny siliques. Plant at right is normal.

soft basal decay (7). Verticillium wilt of stock is reported from Europe but apparently is unknown in the United States; it might produce symptoms similar to those of fusarium wilt.

The wilt *Fusarium* was readily and consistently cultured from the stems of affected plants gathered in the field. Siliques from affected stems were surface treated with sodium hypochlorite before being opened, and the seeds, removed aseptically and placed on agar, regularly yielded the same organism. Commercial seed from these fields also carried this *Fusarium,* along with other fungi. Pathogenicity for *Mathiola* of isolates from all of these sources was confirmed. All plants grown in infested soil at 21°–24° C. developed the disease. Almost all the seed developed on diseased plants 10–12 inches high grown in infested soil in greenhouse pots yielded

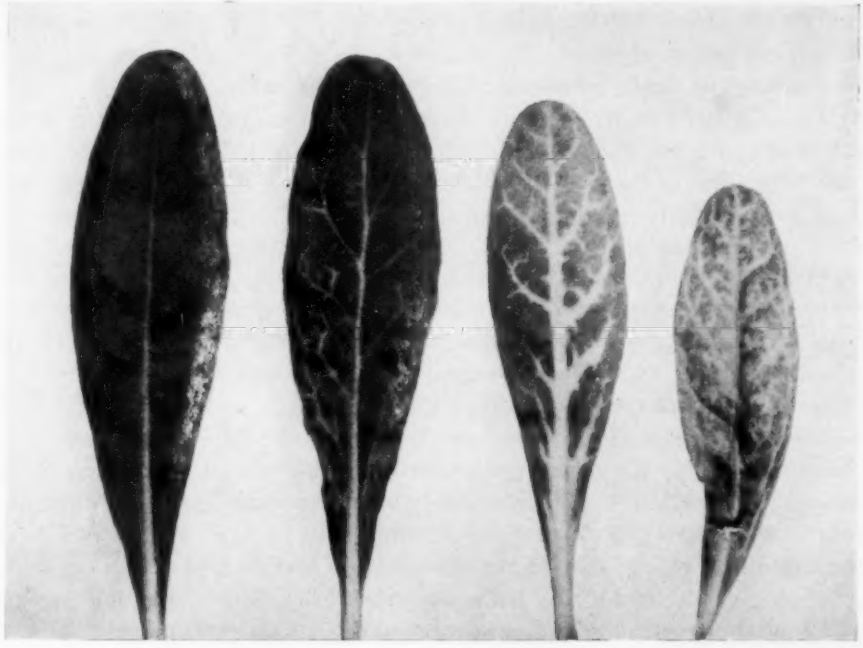


FIG. 2. Lower leaves of column stock plant infected with fusarium wilt. Normal leaf on left.

the *Fusarium* when placed on agar. The conditions of growth of these plants precluded the possibility of fungus penetration through the wall of the silique, and indicated that the fungus grew up through the vascular elements. Not all of these seeds were sufficiently plump to pass through commercial cleaning and milling operations. The pathogen is clearly seed borne, probably internally.

Pathogenicity of the various isolates was readily demonstrated. Single-spore cultures were used in all cases and the fungus inoculum was grown on potato-dextrose broth. The washed mycelial mats were chopped up and

mixed with pasteurized soil in pots into which the stock seedlings were transplanted. The greenhouse chamber was kept at 21°–24° C., and at these temperatures strong symptoms developed. None of the checks was diseased.

The organism was identified by W. C. Snyder as *Fusarium oxysporum* Schl. emend. Sny. and Hans. (6). Two forms of this species, *F. oxysporum* f. *conglutinans* (Wr.) Sny. and Hans. on cabbage and kale (2, 6), and *F. oxysporum* f. *raphani* Kend. and Sny. on radish (4), have been reported on cruciferous crops. Unfortunately, virulent cultures of the kale and radish isolates were not available for test, but a fresh isolate of the cabbage form was obtained. Flats of pasteurized soil were inoculated on April 30, 1947, with mycelial mats of the different forms. The flats were planted with transplants of column stock, Copenhagen Market cabbage, and Dwarf Green Curled kale, and seeds of Early Scarlet Globe radish. Data were taken on June 12. Stocks in the flats with the stock isolates were nearly all dead, but those in flats with the cabbage form were unaffected. Cabbage showed typical yellows with the cabbage *Fusarium*, but no symptoms with the stock isolates. Kale was unaffected by the stock organism, but several of the plants showed typical yellows with the cabbage form. Radish was unaffected by the stock isolates, but showed slight vascular streaking with the cabbage form. All of the positive results reported were confirmed by isolations from vascular tissue. These results indicate that this is a specialized form of *F. oxysporum* which differs biologically from those previously reported on cabbage, kale, and radish. Accordingly, the specialized form which causes vascular wilt of stock, *Mathiola incana*, is designated as *Fusarium oxysporum* f. **mathioli** n.f.[1]

Because a temperature below 18° C. is required for initiation of flower buds of stock (5) the flower crop is grown either under glass or outdoors, depending on the area, during the winter months. Since the vascular fusarium diseases are favored by warm soils (24°–25° C.), it is understandable that this disease has only once been observed in garden or florist plantings, even though the pathogen must have been disseminated commonly with the seed. If a successful summer-flowering stock should be developed, the disease might prove a limiting factor in its use, as has fusarium wilt of China aster.

The California seed crop is sown in the field in December or January, and the seed is harvested the following September or October. Because of this prolonged growth period, the plants mature in the warm months during which the disease appears.

Several different varieties of Ten Weeks, column, and branching stocks have either been observed with the disease in the field or successfully inoculated. It is not known whether any varieties are resistant to this disease.

[1] The spelling follows the correct name of the host, *Mathiola*, rather than the commonly used *Matthiola*. *See* L. H. Bailey. Hortus, p. 8. Macmillan Co., New York. 1930.

SUMMARY

An important vascular fusarium wilt occurs on maturing stock plants (*Mathiola incana* and *M. incana* var. *annua*) in California seed fields, causing premature leaf drop, stunting, vascular discoloration, and development of flattened, faded, light tawny siliques. In infested warm soil, seedlings have conspicuous vein clearing, stunting, and wilting, and they die early.

The stock *Fusarium* is non-pathogenic to cabbage, kale, and radish, and the cabbage form does not infect *Mathiola*. The pathogen is designated as *Fusarium oxysporum* f. *mathioli* n. f. The organism commonly is seed borne.

Since stock requires low temperatures for bud initiation and is grown in the winter months, the disease may prove to be important only in the seed crop which continues growth through the summer.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
LOS ANGELES 24, CALIFORNIA.

LITERATURE CITED

1. DIMOCK, A. W. The Rhizoctonia foot-rot of annual stocks (*Matthiola incana*). Phytopath. **31**: 87–91. 1941.
2. KENDRICK, J. B. Kale yellows in California caused by *Fusarium conglutinans* Wollenw. Hilgardia **5**: 1–15. 1930.
3. ————, and K. F. BAKER. Bacterial blight of garden stocks and its control by hot-water seed treatment. Calif. Agr. Exp. Sta. Bull. 665. 1942.
4. ————, and W. C. SNYDER. Fusarium wilt of radish. Phytopath. **32**: 1031–1033. 1942.
5. POST, K. Effects of daylength and temperature on growth and flowering of some florist crops. N. Y. (Cornell) Agr. Exp. Sta. Bull. 787. 1942.
6. SNYDER, W. C., and H. N. HANSEN. The species concept in Fusarium. Amer. Jour. Bot. **27**: 64–67. 1940.
7. TOMPKINS, C. M., and C. M. TUCKER. Foot rot of China-aster, annual stock, and Transvaal daisy caused by *Phytophthora cryptogea*. Jour. Agr. Res. [U.S.] **55**: 563–574. 1937.

FIELD CONTROL OF BLIND SEED DISEASE OF PERENNIAL RYEGRASS IN OREGON[1]

JOHN R. HARDISON[2]

(Accepted for publication February 2, 1948)

INTRODUCTION

Blind seed disease of perennial ryegrass (*Lolium perenne* L.) caused by *Phialea temulenta* Prill. and Delacr. was first known to occur in the United States during the winter of 1943. Positive identification was made by Dr. E. O. C. Hyde, Seed Analyst at Palmerston North, New Zealand, who recognized typical macroconidia on Oregon-grown seed sent to him for diagnosis by Mrs. L. A. Kanipe, Seed Analyst in charge of the Federal-State Cooperative Seed Laboratory at Corvallis, Oregon. The first apothecia seen in the United States were found by Mr. O. E. Mikesell, County Agricultural Agent, and a group of farmers on a Linn County farm near Halsey, Oregon, May 12, 1944. Apothecia were first positively identified in Oregon by Dr. George W. Fischer (4, 7).

Preliminary work and observations during the period of the winter of 1943 through the summer of 1944, conducted by Mrs. L. A. Kanipe, Mr. H. H. Rampton, Mr. H. A. Schoth, Dr. George W. Fischer and others, was briefly reported by Fischer (7) and in unpublished reports (4, 5, 6). This preliminary work in Oregon and particularly the published work in New Zealand greatly aided the development of field control in Oregon in connection with the writer's studies which began in September, 1944.

ECONOMIC IMPORTANCE

The detrimental effects of blind seed disease on perennial ryegrass seed make it a very serious disease. Although seeds infected during early development are of light weight and can be mechanically separated, most infected seeds have sufficient weight to prevent their removal from healthy seed in harvesting and cleaning operations (20, 25). There is the further difficulty that infected seed cannot be distinguished easily from healthy seed unless the lemma and palea are removed (14). In germination tests, seed laboratories make no separation of the "blind seeds" from healthy seed. Therefore, severely diseased crops of seed are low in germination and much reduced in value.

Greenall (8) mentions that in New Zealand germinations for two consecutive seasons were as low as one per cent in one case, and in very few

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture and the Oregon Agricultural Experiment Station. Published with approval of the Director of the Oregon Agricultural Experiment Station as Technical paper No. 510. Contribution of the Section of Plant Pathology.

[2] Associate Pathologist, Division of Forage Crops and Diseases.

cases higher than 30 per cent. Greenall (8) states further that farmers in the South Otago district of New Zealand may expect low germinations in perennial ryegrass seed one year in every two or three years.

Neill and Hyde (20) state that losses cause high prices of certified seed and thus severely restrict the establishment of new pastures with superior ryegrass from certified lines. They mention further that scarcity and high price of good lines of seed in New Zealand have greatly restricted the development of export trading.

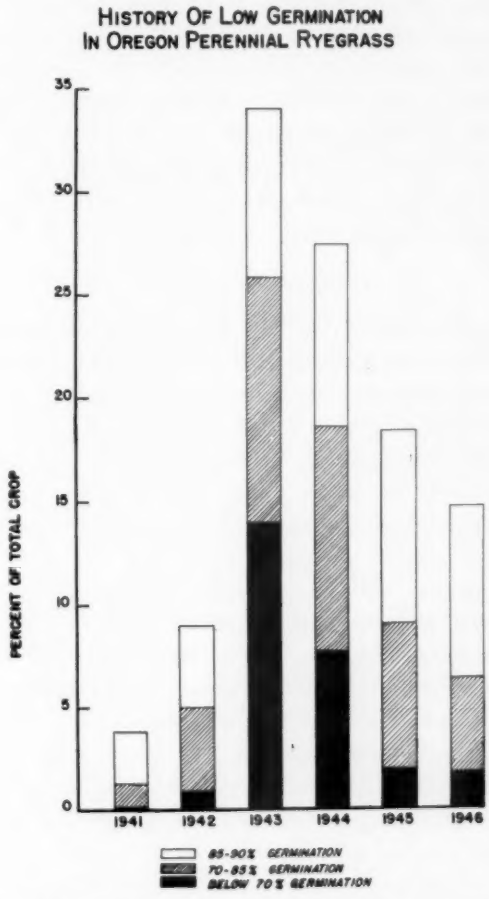


FIG. 1. Bar graph shows history of low germination in Oregon perennial ryegrass Percentages of the total crop are based on individual seed lots.

In Ireland, Calvert and Muskett (1) state that there would be few seasons when by proper blending the seed could not be brought up to a standard of germination suitable for incorporation in mixtures intended for pasture establishment in areas not producing seed. However, this does not prevent severe losses to individual growers.

In Oregon a few cases of low germinating seed appeared in the 1941 crop. Further difficulties were experienced in 1942. The greatest damage occurred in 1943 when one-fourth of the crop could not be certified because of less than 85 per cent germination. Nearly one-seventh of the 1943 crop had less than 70 per cent germination and represented practically unsaleable seed (Fig. 1).

In New Zealand, white clover and perennial ryegrass are often grown as pasture mixtures, and it has been recommended that if the ryegrass seed crop is found badly diseased in preharvest tests the grower may either cut it for hay or perhaps save the field for a later clover seed crop (14, 16). In contrast, perennial ryegrass in Oregon is grown extensively in pure stands primarily for a seed crop; consequently, farmers have no real alternative if the seed crop fails, since returns from ryegrass hay would be only a fraction of that from a good seed crop. Furthermore, a large portion of the perennial ryegrass in Oregon is grown on land that will grow very few other profitable crops. As a consequence, control of blind seed disease is of considerable importance to Oregon agriculture.

LITERATURE REVIEW

Wilson *et al.* (25) review the literature concerning the identity and nomenclature of the blind seed disease fungus. The many contributions to the biology and control of blind seed disease by previous workers may be summarized as follows:

1. Blind seed disease is caused by *Phialea temulenta* Prill. and Delacr., and this is apparently the same fungus previously described from rye (*Secale cereale* L.) in France by Prillieux and Delacroix (22) in 1892 (1, 21, 25).

2. The fungus overwinters in infected seed, producing apothecia during the spring coincident with the flowering of the grass (1, 20, 21, 25).

3. Ascospores initiate the infection, which is followed by a copious production of macroconidia in a slimy matrix on infected seeds that may infect additional flowers (1, 20, 25). Macroconidia produced in artificial culture are equally infectious (20).

4. The seed is the only part of the plant infected, and therefore presence of disease in seed does not affect the value of the grass for pasture, cover crops, or turf (1, 20, 25).

5. Endosperm tissue is permeated by the fungus mycelium and the embryo may often be killed (12, 20, 25).

6. Macroconidia die within a few weeks after harvest and are of no importance in perpetuating the fungus during or after storage of seeds (21).

7. Mycelium in infected seeds dies in natural dry storage within 20 to 24 months (20).

8. The fungus within seeds has been killed by dry heat (21), and by

hot water (1, 2), and partial control has been obtained by the use of chemical dusts (2, 25), but a practical method of seed treatment has not yet been discovered.

9. Preliminary trials indicated that deeper sowing suppresses, at least in part, the development of apothecia, and drill sowing was recommended in preference to broadcasting (25).

10. The disease spreads considerably by air-borne ascospores (1, 20).

11. Perennial ryegrass seeds infected with blind seed disease are nontoxic to animals (21).

12. Methods for examining preharvest head samples and harvested seed samples for the presence of the disease have been described (6, 7, 14, 15, 16, 24, 25).

13. The control suggestions in New Zealand (21) and in Scotland (25) consist primarily as follow: (a) examining crops for infestation before harvest to enable growers to avoid expense of harvesting worthless crops, and (b) planting two-year-old seed or seed from healthy crops.

14. The available control measures have not given satisfactory field control (3, 8).

MATERIALS AND METHODS

A method for rapid determination of the incidence of disease in seed samples, which is a modification of the New Zealand system and the method previously reported by Fischer (7), was described in an unpublished report by Fischer (6). This consisted of mixing equal parts of seed and water, approximately 30 ml. of each, and allowing the seed to soak for about 30 minutes, with occasional shaking, after which the water was separated from the seeds by straining through cheesecloth. A drop of this liquid was then applied to a haemacytometer, and the conidia of the blind seed disease fungus within a specific size division of the counting cell were counted.

In the present studies, this method has been used with only slight modification. Glass vials of approximately 18-ml. capacity are labeled with the seed sample number, and used to measure the seed which is placed together with the same amount of water into a numbered 250-ml. Erlenmeyer flask. These same vials are retained to receive the water strained from the seed after soaking for 20 minutes. A Bausch and Lomb haemacytometer with Neubauer ruling is used to standardize the method. By comparison with the haemacytometer, ordinary microscope slides and cover glasses were found to give reliable results that can be easily interpolated to the haemacytometer readings, if the cover glass is firmly pressed to the slide and care is taken to avoid excess water.

Rose (24) used a similar method by heating 100 seeds in a test tube with 5 ml. water for 15 min. at 80° C., after which the tubes were shaken for half a minute. A drop of the solution was then taken for a conidial count with a Zeiss Thoma haemacytometer and the number covering the 16 large

squares registered. Rose (24) concluded that the method cannot be used for harvest forecast purposes, and he gave preference to Hyde's (14) method of examining individual seeds for infection.

In the Oregon program current harvest forecasts have been largely discontinued, and the method now used appears to be satisfactory for advising growers of the possibilities of the next year's crop based on the probable increase in disease and consequent seed damage.

Since it has been necessary to indicate the severity of disease in seed samples to growers in ordinary terms, the classification shown in table 1 was arbitrarily designed. In this paper all references to degree of infection are based on this classification, which has functioned satisfactorily and is still being used.

TABLE 1.—*Classes of infection for blind seed disease of perennial ryegrass based on tests of cleaned seed samples*

Name of class	Macroconidia per 1/160 cu. mm. [a]	Two-year average germination for class	Field recommendation for next crop
	Number	*Per cent*	
1. None	0	93.0	Safe for harvest
2. Trace	1–3	92.5	Safe for harvest
3. Very Light	4–6	91.5	Should plow if wet field
4. Light	7–15	90.0	Should plow
5. Moderate	16–30	87.0	Plow
6. Moderately Heavy	30–50	79.0	Plow
7. Heavy	50–100	76.5	Plow
8. Very Heavy	100–200	65.5	Plow
9. Extremely Heavy	200–600+	57.0	Plow

[a] Number of macroconidia recovered in water suspension after soaking seed 20 minutes in an equal volume of water.

EXPERIMENTAL RESULTS

Seed Treatment Studies

Results of seed treatment trials planted by H. H. Rampton and H. A. Schoth during the fall of 1943 were reported by Fischer (4, 7). These tests gave no practical control by chemicals or dry heat. Partial control was indicated by a vapor heat treatment conducted for these workers by Miller and McWhorter in the manner they have described (18, 19).

In further trials the writer planted plots in the fall of both 1944 and 1945, using heavily diseased seed treated with proprietary chemicals including Arasan (tetramethyl thiuram disulfide), New Improved Ceresan (ethyl mercury phosphate), Semesan (hydroxy mercury chlorophenol), Spergon (tetrachlorobenzoquinone), Purex (sodium hypochlorite), Yellow Cuprocide (yellow cuprous oxide), and liquid formaldehyde. No reduction in numbers of resulting apothecia was noted. The dry heat treatment as described by Neill and Hyde (21) gave a 65 per cent reduction in apothecia with only a slight reduction in germination. A vapor heat treatment per-

formed by Miller and McWhorter (19) consisting of an approach period of 20 min. at 130° F. followed by 30 min. at 145° F., gave complete control of the fungus with no reduction in germination.

Because sufficient disease-free seed and two-year-old seed have been available to meet the needs of growers for planting new fields for seed production, seed treatment is regarded as impractical in Oregon, and further trials have not been made.

Aging of Seed

Neill and Hyde (21) first reported that no apothecia were obtained from two-year-old seed. Based on this information two-year-old seed was recommended to Oregon growers during the winter of 1943 in a report prepared by H. H. Rampton and Mrs. L. A. Kanipe (23).

The writer planted two-year-old seed in the fall of 1944 and 1945. None of this seed produced apothecia the following spring. On the other hand, in two years' trials, diseased seed, three and 15 months old, planted in the fall (October) produced abundant apothecia the next spring.

The first suggestion of control by spring planting was mentioned by Neill and Hyde (20) and later suggested by Fischer (4, 7). However, observations were incomplete, since observations were made only during the same calendar year diseased seed was planted and not after the seed had been exposed to winter temperatures.

In the writer's studies diseased seed 10 months old planted in the spring (April and May) has produced abundant apothecia the following spring in amounts equal to that obtained from fall planted trials with the same seed. In contrast, samples of heavily diseased seed 22 months old planted in May, 1945, produced no apothecia in 1946.

Based on the above information, Oregon seed growers have been advised that diseased seed is safe for fall planting when past 24 months old (9, 10, 11) and for spring planting when 20 to 22 months old (10, 11).

Depth of Planting Studies

Wilson *et al.* (25) mentioned that experimental evidence indicated that deep planting at least partially suppressed apothecia arising from the seed planted. In the writer's trials during the winter of 1944, seeds buried one-fourth inch or more in moist sand in laboratory tests produced no apothecia, although apothecia were abundant on surface seeds. A field planting was made in October, 1945, with an ordinary grain drill. The soil had been summer fallowed and permitted excellent coverage of seed. No apothecia were obtained in the spring of 1946 from seed buried one-half inch or more.

One farmer in Linn County inadvertently drilled heavily diseased, 1942 crop seed (68 per cent germination) at a depth of 2½ to 3 inches during September, 1943. The field produced two excellent subsequent crops, 97

per cent germination with a trace of disease in 1944, and 95 per cent germination with a very light infection in 1945. There can be little doubt that the accidental deep planting was responsible for the small amount of disease in this instance.

Planting seed at least one-half inch deep or more with complete soil coverage has been recommended in Oregon as an additional safeguard (10, 11).

Chemical Soil Treatments

Fischer (5) suggested the application of chemicals to suppress apothecia in infested fields. In May, 1945, the writer applied 24 different chemical treatments to plots in a heavily infested field near Peoria, Oregon. The treatments included the following chemicals as sprays: Special Semesan (hydroxymercurichlorophenol and hydroxymercuricresol), mercuric chloride and mercurous chloride (1 : 2 mixture), copper sulfate, copper oxide, basic copper sulfate, Sinox (sodium dinitro ortho cresylate), basic Sinox (Sinox plus sodium hydroxide), Puraturf (phenyl mercuri triethanol ammonium lactate), sodium arsenate, Isothan Q15 (lauryl isoquinolinium bromide), and lime-sulfur. Chemicals applied in dry form were Calcium cyanamid (calcium cyanamide), dicyandiamide, sulfur, crude naphthalene, and Dithane A-10 (sodium ethylene bisdithiocarbamate).

Most of these chemicals injured apothecia which were present at the time of application; however, no chemical was effective for more than a short period. Calcium Cyanamid at 150 lb. per acre completely suppressed the apothecia for three days, but normal apothecia were abundant after eight days. The other treatments gave no control or had not completely suppressed apothecia three days after application, and abundant apothecia were noted after eight days. Considering that perennial ryegrass plants are in anthesis over a period of several weeks, these soil treatments did not have practical value. Further trials were omitted because records for the 1945 crop indicated that control had been obtained from the program in operation (Fig. 1).

Trials with Head Sprays and Dusts

During the summer of 1945, flowering spikes of perennial ryegrass in a heavily infested field were given a single application with seven sprays: Puraturf, Isothan Q15, Dithane D-14 plus zinc sulfate and lime (sodium ethylene bisdithiocarbamate), Fermate (ferric dimethyl dithiocarbamate), Sinox-D (ammonium dinitro ortho cresylate), lime sulfur, and Bordeaux mixture 5–5–50, and two dusts: sulfur and Sinox (15 per cent dinitro ortho cresylate). These materials were applied May 25, 1945, when a maximum number of flowers were in anthesis. No reduction in amount of disease was noted for any of the treatments, and further trials have not been made. Corkill and Rose (3) also reported no value from spraying ryegrass plants during the infection stage with various chemical sprays.

Burning as a Control Measure

Perennial ryegrass fields are sometimes burned to remove excessive straw left after combine seed harvesting. One 600-acre field located in Linn County, Oregon, which in 1944 produced a heavily infected crop with 48 per cent average germination and two years' straw accumulation, was burned after harvest in August, 1944. After burning, only a few seeds could be found in the entire field. In the spring of 1945 apothecia were present only in a small area that had been poorly burned. The 1945 crop showed only a trace to very light amount of disease with an average of 97 per cent germination. Three other diseased fields with germinations between 73 and 93 per cent in 1944 were burned after harvest. All three produced seed that germinated above 90 per cent in 1945.

A comparison of the results from burning, in table 2, with the records on similar fields that were not burned, shown in table 3, definitely indicates that burning will reduce blind seed disease and subsequent damage to seed crops. The variation in the reduction of disease shown by the four fields in table 2 is due primarily to the differences in the effectiveness of different fires. The intensity of the burning as evidenced by the completeness of removal of crop residue was best in field A and in decreasing degrees in the order D, B, and C.

TABLE 2.—*Effect of burning straw and stubble of perennial ryegrass, after the 1944 harvest, on blind seed disease and seed germination in the 1945 crop*

Field	Acres in field	1944 crop (before burning)		1945 crop (after burning)	
		Average germination	Amount of disease	Average germination	Amount of disease
	Number	*Per cent*		*Per cent*	
A[a]	618	48	Extremely Heavy[b]	96	Very light
B	212	73	Very Heavy	95	Light
C	200	90	Moderate	95	Light
D	12	93	Light	94	Trace

[a] Field had two years' straw accumulation resulting in a hot, clean burn.
[b] See Classes of infection described under Materials and Methods.

Burning as a remedial control measure is recommended in Oregon only for fields with a marginal infestation after the first or second year's crop. Older fields generally become weedy and seed yields drop. It has been recommended (10, 11) that such fields be plowed to clean up the field and to save the organic matter in the crop residues in preference to burning.

Preharvest Tests for Disease

The principal control measure in foreign countries is the testing of preharvest samples for disease so that growers can avoid harvesting worthless

crops. Unfortunately this system does not insure the farmer a return from his land as far as seed is concerned, and, more important, it leaves heavily infested fields as sources of abundant inoculum for continued infection in old fields and possible infestation of new fields.

Preharvest testing was started in Oregon during the summer of 1944 under the auspices of Federal-State Cooperative Seed Laboratory at Corvallis, with the help of Dr. G. W. Fischer. At first the New Zealand method described by Hyde (14) was used, but Fischer (4, 6, 7) developed a faster method relying on the number of macroconidia recovered from soaking chopped up heads to indicate severity of infection. At first (4, 7) a centrifuge was used to separate the conidia, but later (6) a haemacytometer was used to count the conidia in the water residue.

Preharvest testing was discontinued as a regular practice in Oregon during the fall of 1944 and was replaced by the seed testing service.

Absence of Disease in Regrowth Heads

In 1945 and 1946, samples of regrowth heads which developed in heavily infested fields after harvest were carefully examined. No blind seed disease has been found in regrowth heads. This indicates that no increase of disease potential occurs in a field after the regular harvest.

Oregon Program for Seed Inspection and Field Recommendation

Since the control measures proposed by other workers failed to provide satisfactory field control according to Greenall (8), further measures were thought necessary to control the disease in Oregon. The most apparent weakness in foreign control programs appeared to be the failure to make some disposition of badly diseased fields. Therefore, during the winter of 1944, the writer set up a program for examination of seed samples from every perennial ryegrass seed production field in Oregon entered for certification. A portion of the bulk seed from the certification samples was made available for the disease tests. From the disease tests reports were prepared for all growers, listing the amount of disease found and giving recommendations for field control.

The problem immediately arose to determine the degree of infection in seed samples that would make a field unprofitable for another year's seed production. It was learned from case histories that seed from many fields had dropped from above 90 per cent germination to below 85 per cent in the next crop. Table 3 shows the records for two seed crops from nine perennial ryegrass fields. These records were selected as typical examples from the available case histories and illustrate the approximate increment in disease that may be expected in an infested area. From these records it is apparent that the disease can build up in a field, without noticeably reducing germination, to a point where severe damage will result the following year.

After conducting numerous tests for incidence of disease and comparing the results with the germination of respective seed samples, the eight classes of infection described under Materials and Methods were designed to classify individual samples. The degree of infection in seed samples that would result in a germination below 85 per cent in the next crop was estimated to be the Light class of infection (Table 1). Fields with more disease, *viz.*, Moderate, Heavy, Very Heavy, or Extremely Heavy infestations were regarded as unprofitable for another year, and the recommendation was made (9, 10) that such fields be plowed before May 1, since apothecial production begins in early May. Fortunately this recommendation was rather generally followed. Growers who did not follow these directions invariably produced low quality seed during 1945 and 1946. The plowing of a majority of the badly infested fields has prevented direct loss to growers concerned and has greatly reduced the inoculum available for infestation of new fields. This is especially important, since most new fields have been planted with either disease-free or two-year-old seed since the fall of 1944.

TABLE 3.—*Increment of blind seed disease in nine selected perennial ryegrass fields with different degrees of infection. Fields located in Linn County, Oregon*

Field	1944 crop		1945 crop	
	Percentage germination	Disease infestation	Percentage germination	Disease infestation
A	93	None seen	95	Trace
B	96	Trace	92	Very Light
C	95	Trace	90	Light
D	95	Very Light	90	Moderate
E	91	Light	85	Heavy
F	91	Light	81	Heavy
G	92	Light	76	Very Heavy
H	85	Moderate	72	Extremely Heavy
I	88	Moderate	38	Extremely Heavy

For the fields having a Light amount of disease in the seed test, the information given was that the next year's crop would be a gamble, and that the field should be plowed. It was explained to growers (9, 10) that they would profit by cleaning up such fields and returning them to production of high quality seed as early as possible instead of taking a chance of heavy loss for the year; and also that badly infested fields would constitute serious hazards to neighboring fields in the meantime. The majority of growers with such fields decided to plow them, especially after the 1945 crop results had indicated general success from the recommendations (Fig. 1).

As shown in table 1, perennial ryegrass seed may have a germination of 90 per cent or higher, yet may have considerable blind seed disease infection. Therefore, fields that have produced high germination seed cannot necessarily be regarded as safe, since such fields may have a Light

or Moderate disease infestation and may produce a crop of low germination seed the following year. A spectacular example was a field near Halsey, Oregon, that produced seed of 90 per cent germination but with a Light disease infestation in 1944. The 1945 crop had an average germination of 38 per cent with an Extremely Heavy infection of blind seed disease.

Fields with no disease, a Trace, or a Very Light amount of disease were regarded at first as probably safe for another year's production. Although many fields with a Very Light infestation have produced seed the next year that averaged well above 90 per cent germination, a number of fields on wet lands with this degree of infestation have produced seed of poor germinability. As a result the recommendations have been modified, and it is now suggested that fields with a Very Light infestation be plowed if the field is poorly drained and likely to be wet in May and early June.

In making recommendations to County Agricultural Agents, it is suggested that, when advising growers, they should give attention to local conditions, such as age of fields, presence or absence of infested adjoining fields, burning, and other factors that might influence the expected disease infestation.

During the three years the program has been in operation, there has been a gradual elimination of diseased fields, and an increase in fields without disease. In the 1946 crop 52 per cent of the fields had no disease as compared with 27 per cent in 1945 and 13 per cent in 1944. If this trend continues, the disease eventually may be almost completely eliminated as a limiting factor in seed production of perennial ryegrass in Oregon.

Disease-Free Seed Stocks

A valuable by-product of the examination of all samples of Oregon perennial ryegrass is information that makes possible the selection of disease-free seed lots for planting new seed producing fields. Seed lots having no disease, 95 per cent germination, and blue tag purity have been selected and approved from each crop starting with 1944. This has provided growers with a source of officially approved seed and has contributed materially to the success of the control program. The supply of such seed was limited in 1944 but greatly increased in 1945. In 1946, more seed stock of this quality was available than was needed for reseeding. Similarly Calvert and Muskett (1), have recommended that the best seed be kept for distribution in seed-producing areas of Ireland.

Evidence of Control

The data illustrated by the bar graph in figure 1 indicate that good control of blind seed disease in Oregon was obtained in 1945 and 1946. The improvement for the 1944 crop in comparison with 1943 appears to have been due in part to dryer weather conditions. Also, farmers plowed most fields that produced low germination crops in 1943. In addition, some

attention was given to planting high quality seed in the fall of 1943. A substantial part of the 1944 crop was cut for hay and not harvested for seed because of the recommendations from the Experiment Station based on preharvest tests. This reduced the amount of badly diseased seed entered for certification, and consequently affected the data in figure 1. The improvement in the 1945 and 1946 crops appears to be justifiably credited to the control program put into practice during the winter of 1944. Using 1943 as a year very favorable for blind seed disease, a comparison of weather records for the Willamette Valley indicates that 1944 was relatively unfavorable and that 1945 and 1946, while less favorable than 1943, were much more favorable than 1944 for blind seed disease devolopment. The large amount of badly diseased seed produced in the unfavorable 1944 crop year suggests that the decided improvement in Oregon perennial ryegrass in 1945 and 1946 should be credited primarily to the control program described in this paper.

The control measures that are recommended in Oregon (9, 10, 11), may be summarized as follows:

1. Plant disease-free seed approved by the Experiment Station.
2. Recommendations on planting diseased seed:
   A. Diseased seed is safe for fall planting only when more than two years (25 to 27 months) old. Seed 15 months old is as dangerous as that three months old when fall planted.
   B. Spring planting of diseased seed is safe when seed is 20 to 22 months old. Diseased seed from the previous harvest, 9 to 11 months old, is not safe for spring planting.
3. Plant seed in all seed production fields at least one-half inch deep with complete soil coverage as an extra margin of safety. Good seedbed preparation facilitates this practice.
4. Plow up badly infested fields when notified by the Experiment Station. Plowing should be done before May 1 to prevent emergence of apothecia and consequent danger to nearby fields.
5. Preharvest tests are justified only for those fields indicated as marginal in the seed test.
6. Cultural practices that will reduce the disease are as follows: Pastures may be a source of infestation; therefore, it is recommended that pastures should be planted with disease-free or two-year-old seed, and that no heading in pastures be permitted until after July in seed producing districts. Clean plowing is desirable to bury the blind seeds below the furrow slice if possible. Good soil drainage would be helpful, since apothecial production is favored by wet soils.

DISCUSSION

Blind seed disease of perennial ryegrass has been successfully controlled in Oregon by the control program described in this paper. A comparison

of the Oregon program with programs in foreign countries suggests that one of the main reasons for lack of control elsewhere has been the failure to eliminate infested fields. Because of the natural spread of the disease by ascospores, the excellent control measure consisting of planting disease-free or properly aged seed cannot provide more than partial control when the disease is left unchecked in nearby fields. This situation is acknowledged by Neill and Hyde (20) who suggest the development of resistant varieties as the most hopeful method for control. The situation, as it appeared in 1943 the world over, was well described by Greenall (8) who stated that much is known about the disease but little about its control. Corkill and Rose (3), as late as 1945, reported that none of the suggested control methods appeared to be completely effective in controlling the disease in a season favorable for disease development.

Since there is a fundamental difference between culture of perennial ryegrass in Oregon and in foreign countries, it may not be practical to operate elsewhere the plan as developed in Oregon. In foreign countries perennial ryegrass apparently is grown primarily for pasture, with seed production a secondary proposition. Greenall (8) states that seed production in New Zealand depends on wet weather furnishing abundant pasture so that part of the plantings can be closed up for seed production. In contrast, in seed harvest areas of Oregon perennial ryegrass is grown primarily for seed production, and pasture use of seed fields, while important, is a secondary utilization. This striking difference makes it easy to put into operation the plan described under the Oregon program and probably would make it difficult to operate in foreign countries. The plowing up of infested but otherwise good forage producing fields would appear to be difficult to obtain in New Zealand, especially when the risk of seed production is not too good.

Under these circumstances, it is difficult to determine how the disease could be controlled satisfactorily in other countries unless by resistant varieties or by designating special districts for seed production and others for forage as suggested by Hyde (12).

Hyde (12) suggested that the more arid districts must be looked to for quality seed production in New Zealand. This may be a good plan. It is noticeable that the disease has been slow to penetrate into the drier areas in Oregon. This, however, probably will not be an important factor in Oregon because most of the ryegrass seed is produced and probably will continue to be produced on land with poor drainage, making it very favorable for disease development. Climatic conditions appear to favor the disease nearly every year over a large part of the seed production area.

The pre-harvest testing plan for control as practiced in foreign countries is not adapted to Oregon conditions and was discarded early in these investigations. This plan which involves the detection and cutting of worthless crops for hay or waiting for a later clover seed crop invites difficulties

the following year, since this procedure leaves the maximum amount of diseased seed in a field. The disease potential is very high, and if soil moisture is sufficient to permit production of apothecia, the crop may be a total loss. Since in New Zealand (8) much of the seed production depends on abundant rainfall, the production of good quality seed is always jeopardized.

Under the Oregon program for inspection of seed samples, farmers are advised months in advance of the next harvest whether or not they can expect a good crop. Worthless crops can be avoided and infested stands destroyed long before harvest, permitting the farmer to produce other crops or fallow his land. At the same time, other fields are protected by this removal of heavy inoculum that would otherwise be disseminated. The owners of safe fields have increased assurance of good crops. This program has nearly stabilized the perennial ryegrass seed producing industry in Oregon as far as seed germination is concerned.

In controlling this disease, consideration must be given to the fact that the production of seed of perennial ryegrass, in common with that for most other forage crops, normally returns a relatively low acre income. Therefore, most of the control to be practical must be derived through cultural practices. Fortunately, the successful control program in Oregon consists largely of proper cultural practices with close attention to the developmental history of disease in each field. The service to growers, consisting of seed inspection and field recommendation, is in demand and is being adopted as a function of the Oregon Experiment Station.

Seed treatment tests failed to provide satisfactory control in Oregon. This substantiates findings elsewhere. However, ample amounts of aged seed and disease-free seed have been available to meet the needs of Oregon farmers for planting new seed production fields.

Trials on suppression of apothecia and protective head treatments did not meet with practical success. This approach to the problem is regarded as impractical, and further trials have not been made.

Planting seed deep to prevent emergence of apothecia, as first suggested by Wilson *et al.* (25), has met with general success in both laboratory and field trials and has been further verified by field observations. Planting seed in all new seed fields at least one-half inch deep with complete soil coverage has been recommended in Oregon for 1945 and 1946 (9, 10) as an extra precaution against spread of the disease.

Burning stubble and straw residue has given good control in badly infested fields. However, in Oregon, burning is regarded as a drastic treatment and is recommended only for fields that have become severely infested during the first or second year. It is suggested that older fields be plowed because yields are generally declining and weeds are usually becoming a serious problem (9, 10).

The disease has not been found in regrowth heads that invariably form after harvest in perennial ryegrass fields. This suggests that there is no increase of the disease in a field after the regular seed harvest in Oregon.

The investigations in Oregon have been in large part, by necessity, a matter of field experimentation on a very large scale. By a study of case histories it was possible to predict fairly well the course of events in specific fields. The program involving recommendations to farmers is still subject to improvement and probably will be further refined to meet changing conditions. The success so far attained suggests that the program is worthy of trial in other countries.

SUMMARY

1. Blind seed disease of perennial ryegrass has been controlled successfully, apparently for the first time, in fields in Oregon. Control is based on the elimination of badly diseased fields through a program of inspection of seed samples from the previous crop from all fields and recommendations developed for growers.

2. Experimental work indicated the following:

Seed treatment, protective head sprays and dusts, and chemical soil treatments to suppress apothecia are not of practical value.

Aging of seed eliminates danger of spread by seed.

Seed planted one-half inch or deeper with complete soil coverage prevented emergence of apothecia.

Burning straw and stubble after harvest controlled the disease for at least one year.

The disease has not been found in regrowth heads produced after the regular seed harvest.

3. Preharvest testing as a control measure is regarded as impractical in Oregon and has been replaced by the program of inspection of seed samples.

OREGON AGRICULTURAL EXPERIMENTAL STATION,
CORVALLIS, OREGON.

LITERATURE CITED

1. CALVERT, E. L., and A. E. MUSKETT. Blind seed disease of rye-grass. Nature (London) 153: 287–288. 1944.
2. ————, and ————. Blind-seed of rye-grass (Phialea temulenta Prill. and Delacr.). Ann. Appl. Biol. 32: 329–343. 1945.
3. CORKILL, L., and R. E. ROSE. Observations of susceptibility of perennial ryegrass to blind-seed disease. New Zealand Jour. Sci. and Tech. (Sec. A) 1: 14–18. 1945.
4. FISCHER, GEORGE W. The blind-seed disease of ryegrass (Lolium spp.) in Oregon. Unpublished report covering trip to Oregon May 30 to June 8, 1944.
5. ————. Second report on investigations of the blind-seed disease of perennial ryegrass in the Willamette Valley, Oregon. Unpublished report covering trip to Oregon June 17 to June 28, 1944.
6. ————. Detection of blind-seed disease in seed samples of English ryegrass. Unpublished report covering special investigations mainly by the Oregon Cooperative Seed Laboratory and others, July to September, 1944.
7. ————. The blind-seed disease of rye-grass (Lolium spp.) in Oregon. (Abstr.) Phytopath. 34: 934–935. 1944.

8. GREENALL, A. F. Low germination of perennial rye-grass seed in South Otago. New Zealand Jour. Agr. **67**: 79-81. 1943.
9. HARDISON, JOHN R. Blindseed disease and plans for control. Oregon Seed Growers League, Proc. Fourth Annual Meeting (Jan., 1945), pp. 15, 17, 19–20. 1945.
10. ————. A progress report on blindseed disease of perennial ryegrass in Oregon. Oregon Seed Growers League, Proc. Fifth Annual Meeting (Jan., 1946), pp. 71, 73, 75–78. 1946.
11. ————. Present status of the blindseed disease problem. Oregon Seed Growers League, Proc. Sixth Annual Meeting (Dec., 1946), pp. 63, 65, 67, 68. 1947.
12. HYDE, E. O. C. Germinating capacity of perennial rye-grass seed. New Zealand Jour. Agr. **44**: 316–319. 1932.
13. ————. Germinating capacity of Italian ryegrass seed. New Zealand Jour. Agr. **56**: 357. 1938.
14. ————. Detecting Pullularia infection in ryegrass seed crops. New Zealand Jour. Agr. **57**: 301–302. 1938.
15. ————. Examination of ryegrass seed for blindseed disease. New Zealand Jour. Agr. **65**: 349–350. 1942.
16. ————. Examination of ryegrass seed for blindseed disease. New Zealand estimation of germinating capacity. New Zealand Jour. Agr. **70**: 271-276. 1945.
17. MUSKETT, A. E. and E. L. CALVERT. Blindseed disease of ryegrass. Nature (London) **146**: 200–201. 1940.
18. MCWHORTER, F. P., and P. W. MILLER. The application of vapor-heat as a practical means of disinfesting seeds. (Abstr.) Phytopath. **34**: 935. 1944.
19. MILLER, P. W., and F. P. MCWHORTER. The use of vapor-heat as a practical means of disinfesting seeds. Phytopath. **38**: 89–101. 1948.
20. NEILL, J. C., and E. O. C. HYDE. Blindseed disease of ryegrass. New Zealand Jour. Sci. Tech. (Sec. A) **20**: 281–301. 1939.
21. ————, and ————. Blindseed disease of ryegrass, II. New Zealand Jour. Sci. Tech. (Sec. A) **24**: 65–71. 1942.
22. PRILLIEUX, EDOUARD E., and G. DELACROIX. *Philalea temulenta* nov. sp. Prill. et Delacr., etat ascospore *d'Endoconidium temulentum*, champignon donnant au seigle des propriétés vénéneuses. Bull. Soc. Myc. France **8**: 22–23. 1892.
23. Report of the Grass Seed Committee. Oregon Seed Growers League, Proc. Third Annual Meeting (Jan., 1944), p. 27. 1944.
24. ROSE, R. E. Germination and conidial number relationship in blind-seed disease. New Zealand Jour. Sci. and Tech. (Sec. A) **27**: 255–257. 1945.
25. WILSON, M., M. NOBLE, and E. G. GRAY. Blindseed disease of ryegrass, and its causal fungus. Trans. Roy. Soc. Edinb. **61**: 327–340. 1945.

BOOK REVIEWS

WOLF, FREDERICK TAYLOR. *The Aquatic Oomycetes of Wisconsin.* Part 1. 64 pp, 52 fig. The University of Wisconsin Press, Madison, Wisconsin, 1944. $1.50.

In the present confused state of fungus classification and nomenclature, the determination of species remains a difficult task requiring the attention of specialists in particular groups. The task is brought within the range of the general mycologist, however, with the appearance of local floras of this type, in which the literature is organized, nomenclatural problems are reduced, and the work of identification simplified because of the limited number of species to be considered. A local flora is of value only to the extent that it is based upon critical study of a sufficient amount of material to assure a reasonable degree of completeness. Wolf's study of fifty-five species in twenty-two genera of the Lagenidiales, Blastocladiales, Monoblepharidales, Saprolegniales, Leptomitales, and Pythiales appears to be satisfactory in this respect. It includes several species of pathological importance. The descriptions and illustrations of species and the keys to facilitate their identification should be a welcome aid to mycologists and pathologists in Wisconsin and adjacent areas, while the introductory discussion and the extensive list of literature should find even wider interest. More specific information on the extent of the survey upon which the work is based would make an estimate of its probable completeness more precise. Also, a general discussion of habitats and ecological relationships might be a desirable addition. There must inevitably be omissions in an initial survey of such obscure organisms. Nevertheless, the work is an important contribution and stimulus to the production of a comprehensive flora.—E. S. LUTTRELL, Department of Botany, University of Missouri, Columbia, Missouri.

DICKSON, JAMES G. *Diseases of field crops.* 401 pp. McGraw-Hill Book Company, Inc. New York and London. 1947. $4.50.

This book presents briefly and concisely the important information on diseases of field crops. It is a revision of "Outline of Diseases of Cereal and Forage Crop Plants of the Northern Part of the United States," published in mimeoprint by Burgess Publishing Company, Minneapolis, in 1939. There has been considerable change in the scope of material presented: diseases of five additional crops have been included, significant new information has been added, and the literature list for each chapter has been revised and brought up to date. In addition, 102 illustrations have been included.

The text is divided into four sections: Part 1, General introduction, includes a brief discussion on physiologic anatomy of certain plants in relation to diseases; Part 2, Diseases of cereals and grasses; Part 3, Diseases of legumes; and Part 4, Diseases of fiber and other field crops. In addition to a good index, the book also contains an appendix in which the diseases are grouped on the basis of the primary causal agents and a second appendix in which the causal organisms are arranged according to order and family.

The crop plants are arranged alphabetically in each section. As in the previous publication, the diseases of each crop are considered in the following sequence: nonparasitic, virus, bacterial, phycomycetous, ascomycetous, hypomycetous, and basidiomycetous diseases. Although detailed discussion of a particular disease is given under one crop only, considerable repetition sometimes appears if the disease occurs on several hosts. This also necessitates frequent cross-references to different chapters. If the book were organized on the basis of diseases, its value as a text book would be materially increased. This would allow for more definite information on certain diseases without increasing the volume of the book. However, the book was intended as a convenient reference outline and I presume many crop specialists would prefer the arrangement on the crop basis.

The more important diseases are discussed from the following standpoints: Geographic distribution, economic importance, symptoms, causal agent (and morphology of the organism), conditions conducive to development, and control measures; while less important diseases usually are presented briefly. Keys for the determination of physiologic races of many of the important pathogens are included.

Many of the illustrations in the book are excellent and valuable for diagnostic purposes, others are poorly reproduced, and a few are worthless. The quality of illustrations probably may be attributed to the type of paper used, and I presume will be remedied as soon as a better quality of paper becomes available. The book is well written and relatively free from typographical errors. The book should be of material value to pathologists and crop specialists everywhere. The author should be commended not only for bringing a vast amount of material together in one volume, but because of his generosity in relinquishing all claims to royalties and thus reducing the price of the book.—J. J. CHRISTENSEN, University Farm, St. Paul 1, Minnesota.

Make your finished dusts well-conditioned . . .

with ATTACLAY!

By using Attaclay as extender or conditioner, finished dust blenders and their grower-customers both benefit.

Blenders take advantage of Attaclay's low bulk density to adjust the bulk of finished dusts. As a result, their whole line of crop dusts has a near-uniform volume. Packages and containers become standardized—mixing procedures streamlined. What's more, they work with a highly adsorptive, free-flowing material that eases many blending steps.

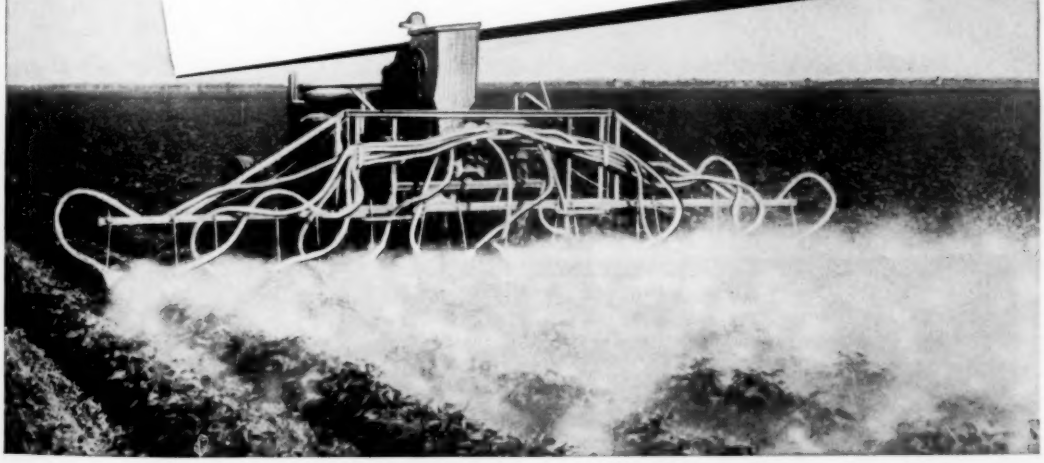
Growers benefit, too. They don't have to constantly adjust their rigs or speeds to compensate for "light" or "heavy" dusts. Besides, many growers know from experience how well Attaclay-mixed dusts will flow, disperse, settle, cover, stick and kill—how its lack of abrasiveness lengthens rig life.

Look into our diluent for *your* finished dust formulations. All the good points that make Attaclay so popular with concentrate makers will work equally well to *your* profit. A generous, free sample is yours for the asking.

ATTAPULGUS CLAY COMPANY

Dept. S, 210 West Washington Square, Philadelphia 5, Pa.

Photo—U. S. Dept. Agri.



Purchase from our advertisers. Mention Phytopathology when answering Ads.

THE VANDERBILT LABORATORIES EAST NORWALK, CONNECTICUT

Aids for Agriculture

Work in these laboratories assures constant improvement and extension of our list of well-known products for agriculture

Carriers
- PYRAX ABB
- CHEROKEE Clay
- CONTINENTAL Clay

Dispersing Agents
- DARVAN #1
- DARVAN #2

Sticker
- VEEGUM

Toxicant
- SODIUM SELENATE

Samples and literature available

R. T. VANDERBILT CO., INC.

Specialties Department

230 PARK AVENUE, NEW YORK 17, N. Y.

Stauffer CHEMICALS SINCE 1885

AGRICULTURAL CHEMICALS

SULPHURS
- Spraying
- Dusting
- Soil
- Burning
- Mixtures

CHLORINATED CAMPHENE

DDT (Dichloro-Diphenyl-Trichloroethane)

BHC (Benzene Hexachloride)

ROTENONE
PYRETHRUM
CALCIUM ARSENATE
NICOTINE
CRYOLITE
COPPER DUSTS
SPRAYING OILS
SOIL NUTRIENTS
TARTAR EMETIC
CARBON BISULPHIDE
BORAX

Also USDA and State approved mixtures of the above insecticides and fungicides.

STAUFFER CHEMICAL CO.

420 Lexington Ave., New York 17, N. Y. 636 California St., San Francisco 8, Cal.
221 North LaSalle St., Chicago 1, Ill. 555 South Flower St., Los Angeles 13, Cal.
Apopka, Fla.—North Portland, Ore.—Houston 2, Texas—Weslaco, Texas

TRIARCH BOTANICAL PRODUCTS

Fine Microscope Slides for Critical Botanists

NOTHING CAN TAKE THE PLACE OF A GOOD MICROSCOPE SLIDE FOR TEACHING PLANT STRUCTURE AS THE BASIS FOR FUNCTION.

TRIARCH slides for plant pathologists are prepared by a plant pathologist who appreciates the necessity of demonstrating the host-parasite reactions clearly. We believe that Triarch Quadruple Stain (safranin, crystal violet, fast green, gold orange) yields an unexcelled differentiation in pathological tissues. Furthermore, as all our slides are stained by the same schedule, the stain gives a comparative differentiation which indicates the relative ages of tissues, degree of disintegration due to fungus advance, and many cytological details. Wherever possible, lesions are selected to show a border of normal tissues for comparison.

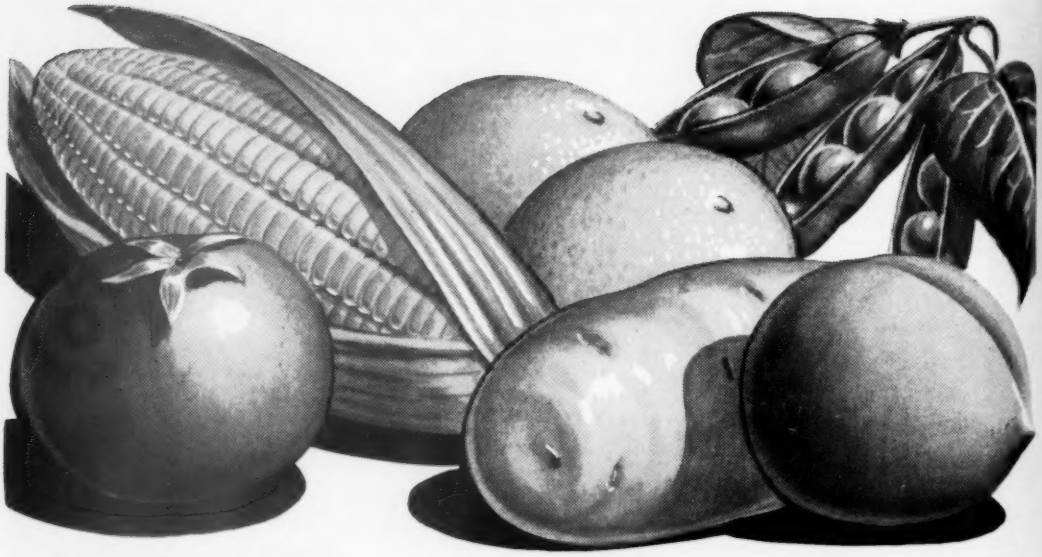
In addition to the considerations listed above, bear in mind the following additional advantages in purchasing Triarch slides:

1. **Largest available collections to choose from.**
2. **Accurate, dependable service, and one price to all patrons.**
3. **Special research assignments accepted, or slides made to order.**

TRIARCH MICROSCOPE SLIDES are used by botanists in nearly 700 educational institutions in every state in the United States and in 18 other countries, whose purchases total more than 100,000 slides each year.

Watch for special announcements in our quarterly publication, TRIARCH TOPICS, and write for a copy of our catalog No. 7 if you do not have it.

GEO. H. CONANT
TRIARCH BOTANICAL PRODUCTS
Ripon, Wisconsin



REAP the PROFITS

.... by producing richer, healthier products, high in vitamin content, and, only soils rich in minerals produce this kind of product. The essential elements of Manganese, Copper, Zinc, Iron and Boron are all found ES-MIN-EL -- Let ES-MIN-EL improve your soil and produce healthier, richer crops for you.

ES-MIN-EL
ESsential MINeral ELements

Write for **FREE BOOKLET**

TENNESSEE CORPORATION
P. O. Box 2205
Atlanta, Ga.

For information on Mineralized Fertilizer, Write
TENNESSEE CORPORATION,
Lockland, Ohio
TENNESSEE CORPORATION,
East Point, Ga.
CAPITOL FERTILIZER CO.,
Montgomery, Ala.

TENNESSEE Atlanta, Georgia — TC TENNESSEE CORPORATION — **CORPORATION** Lockland, Ohio

PHYTOPATHOLOGICAL CLASSICS

A New PHYTOPATHOLOGICAL CLASSIC-No.8

L. J. Tyler, *Bus. Mgr.*
Cornell University
Ithaca, New York

L. Carl Knorr, *Editor*
Citrus Experiment Station
Lake Alfred, Florida

Classic No. 1. FABRICIUS. Attempt at a dissertation on the diseases of plants. 1774. Translated from the Danish by Mrs. Margaret Kølpin Ravn. **(Out of Print.)**

Classic No. 2. FONTANA. Observations on the rust of grain. 1767. Translated from the Italian by P. P. Pirone. Price $0.50.

Classic No. 3. MILLARDET. The discovery of Bordeaux mixture. Three papers. Translated from the French by F. J. Schneiderhan. Price $0.50.

Classic No. 4. WORONIN. Plasmodiophora brassicae, the cause of cabbage hernia. 1878. Translated from the German by Chas. Chupp. Price $0.75.

Classic No. 5. TILLET. The cause of the corruption and smutting of wheat. 1755. Translated from the French by H. B. Humphrey. Price $1.25.

Classic No. 6. PRÉVOST. Memoir on the immediate cause of bunt or smut of wheat. 1807. Translated from the French by G. W. Keitt. Price $0.75.

Classic No. 7. MAYER, 1886; IVANOWSKI, 1892; BEIJERINCK, 1898; BAUR, 1904. Three early papers on tobacco mosaic and one on infectious variegation. Translated from the German by James Johnson. Price $0.75.

Classic No. 8. BERKELEY. Observations, botanical and physiological, on the potato murrain. Also selections from the author's "Vegetable Pathology" made by the Plant Pathology Committee of the British Mycological Society. Price $1.50.

PHYTOPATHOLOGICAL CLASSICS is a series of reprints of outstanding early papers in the field of plant pathology, and embraces titles that are unavailable in most libraries. These classics are of interest not only to pathologists but also to mycologists, plant breeders, physiologists, and other workers in the field of plant science. Additions to the series are made whenever proceeds from the sale of former issues make funds available.

Price of Classics Nos. 2 to 8 inclusive, $6.00. **Please remit with order.**

Published by the

AMERICAN PHYTOPATHOLOGICAL SOCIETY

Address orders to

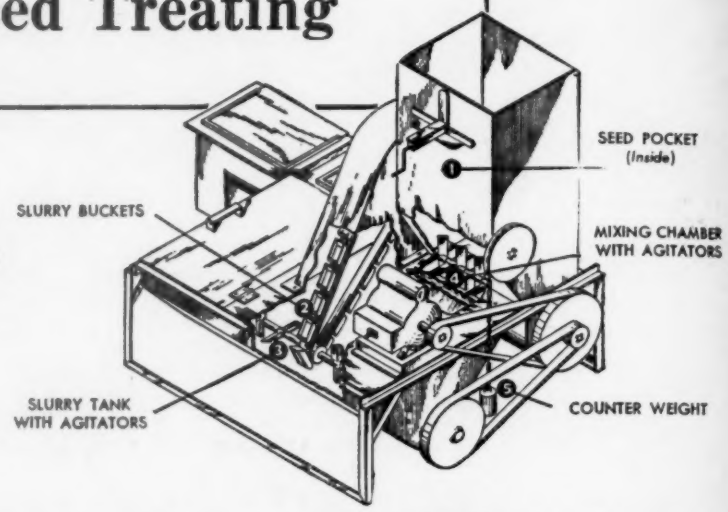
L. J. Tyler, Department of Plant Pathology

Cornell University, Ithaca, New York

Fourth Progress Report on the

Slurry Method of Seed Treating

"CERESAN" M SLURRY METHOD FOR TREATING SEED GRAINS



THE mechanical and biological studies of the slurry method of applying "Ceresan" M to seed grains have been completed. The "Ceresan" M container labels will be revised shortly to provide directions for the treatment of seed grains by both the slurry and the dust methods.

The essential items of information with respect to the slurry concentration and the size of the gate in the seed hopper to be used in treating seed grains are shown at the right. The slurry bucket recommended for all grains is the same as that used for seed corn and has a capacity of 23 cubic centimeters.

Whenever possible, we shall be glad to arrange for a demonstration of the slurry treater to official investigators. For further details, you are invited to write to E. I. du Pont de Nemours & Co. (Inc.), Du Pont Semesan Laboratory, Wilmington 98, Delaware.

THE SLURRY TREATER enables processors of grain and other seeds to treat seed accurately and uniformly. Flying dust is eliminated; seed can be sacked and stored as usual.

SLURRY CHART FOR TREATING SMALL GRAINS

Seed	Average Unit* of Seed	Seed Gate Number	Lbs. "Ceresan" M per Gal. (U. S.)
WHEAT	10.5	15	1.00
RYE	9.5	15	1.00
BARLEY	10.0	18	1.25
OATS	8.5	20	1.50

*Average weight of seed which trips and empties the tilting grain pocket of the slurry treater. Each unit receives 23 c. c. of the "Ceresan" M Slurry.

DU PONT
Seed Disinfectants

"CERESAN" is a registered trademark of E. I. du Pont de Nemours & Co. (Inc.)

BETTER THINGS FOR BETTER LIVING . . . *THROUGH CHEMISTRY*

DU PONT
REG. U. S. PAT. OFF.

Listen to Du Pont "CAVALCADE OF AMERICA"—*Every Monday Night, NBC Network*

Purchasing from our advertisers helps your Journal.